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STUDIES ON TOXOPLASMA AND THE SEROLOGY OF TOXOPLASMOSIS

BY

PAUL GRÖNROOS

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY,
UNIVERSITY OF HELSINKI

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PAUL GRÖNROOS

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To my family

PREFACE

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Grankulla, May 1955.

P. G.

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HISTORICAL SURVEY

In 1908 Nicolle & Manceaux (116) isolated a microorganism from the North African rodent *Ctenodactylus gundi*. They found the microorganism in microscopic preparations of various parts of the rodent. Morphologically it was similar to the protozoa of the Leishmania group, through no kinetoplast could be shown. In 1909 they gave the microorganism the name toxoplasma (117). Their investigation was very accurate; among other things, they noticed the binary, longitudinal division which only much later was assumed to be the reproduction mechanism.

The same year (1909), Splendore (153, 154) described a microorganism similar to toxoplasma which he had observed in the rabbit in Brazil. He claimed to have observed schizogony, and referred the toxoplasma to the Sporozoa class.

It should be pointed out in this connection that as early as 1900 Laveran (102) reported on an organism similar to toxoplasma in the paddy birds *(Padda oryzivora)* of Java. For some reason this publication has not attracted the attention it probably deserves. Wenyon (170), among others, is of the opinion that it is toxoplasma that Laveran described.

Since 1909 spontaneous toxoplasma infection has been observed and reported in a number of different animals. The toxoplasmata isolated from them, considered to represent different species, have been named after the animal species from which they were isolated. The following are found topping a comprehensive list of toxoplasma isolations compiled by Biocca (16), after Nicolle & Manceaux and Splendore: *Toxoplasma canis* 1910, *T. talpae* 1910, *T. columbae* 1911, *T. sporophilae* 1911, *T. sicilides* 1911, *T. neoph-*

rontis 1912, etc., up to *T. caviae* 1916. Fewer cases were reported up to 1930 when a somewhat livelier period started, culminating in *Toxoplasma hominis* 1939 isolated by Wolff & al. (192). Castellani (22) had described a case of toxoplasmosis in man in 1913. He called his species *Toxoplasma pyrogenes.* It was later considered that it was not toxoplasma that Castellani had described, though present opinion has reversed this decision (23). Biocca's list includes 49 animal species from which toxoplasma has been isolated. Hence toxoplasma is of very general occurrence in the animal kingdom. A contributory reason for the high number of toxoplasma species reported is perhaps that the toxoplasma, after Splendore's (154) works, was believed to be a Sporozoon, and the Sporozoa are known to be usually host-specific. All the species were experimentally proved capable of infecting the most heterogeneous homothermal animal species (168). By contrast, it is uncertain whether toxoplasma can infect and reproduce itself in poikilothermal animals (107, 146).

Chatton & Blanc (25) suggested in 1917 that all the species till then considered different toxoplasma species were actually members of a single species. This species, according to the rules of nomenclature must, they said, be called *Toxoplasma gondii,* the name given by Nicolle & Manceaux (117).

All toxoplasma strains isolated since the publications by the above authors have proved to be serologically identical (137). Biologically, however, they vary considerably (49, 87, 183).

For a microorganism to qualify as a toxoplasma it must meet the following requirements indicated by Sabin (139):

1. Typical morphology and staining characteristics of extracellular forms in impression films of fresh tissue or exudate.
2. Pathogenity for mice, guinea-pigs, rabbits, chicks, etc.
3. Obligate intracellular parasitism (143).
4. Immunologic relationship with established strains.

Hence it is impossible to decide on morphological grounds alone whether or not a microorganism is a toxoplasma. Among the organisms that may be thought similar to toxoplasma are: (1) Encephalitozoa, (2) Sarcosporidia, (3) »P-organism», (4) Leishmania, (5) Coccidia, and why not even (6) Malarial plasmodia. Fren-

kel's (50) »P-organism», isolated from Peromyscus, is an organism largely similar to toxoplasma but different in its staining properties and serologically. Encephalitozoon is probably the microorganism most difficult to distinguish from toxoplasma. Perrin (126), however, has shown a number of dissimilarities between this organism and toxoplasma, which is evidence that two different organisms are involved.

The Problem

Epidemiological investigations using Sabin & Feldman's dye test (DT, see p. 31) have shown that toxoplasmosis *can* be a widespread infection.

The parasitologically verified cases of toxoplasmosis have given us a *clinical picture,* that of congenital toxoplasmosis.

Cases of congenital toxoplasmosis are few. The incidence of the infection is generally believed to be very high but unfortunately little is known of toxoplasmosis in adults and children. How great is in fact the number of latent toxoplasma carriers? Despite 15 years of intense study all over the world, the question remains unsolved. The same on the whole can be said to apply to the epidemiology of toxoplasmosis.

Gard (53) asked himself (1953), when comparing poliomyelitis with toxoplasmosis: »Is toxoplasmosis also a civilization disease, destined to become of a greater medical significance as the infection becomes less frequent? Such a possibility is sufficiently alarming to justify serious efforts to ascertain the epidemiology of toxoplasmosis. Thus, a study of its 'social serology' is in the first place called for.»

Similar thinking led the present author to start studying the serological epidemiology of toxoplasmosis in Finland. Work with the microbe toxoplasma and the problems associated with the serological tests for toxoplasmosis provided the results summed up on p. 99.

I. TOXOPLASMA IN GENERAL

Morphology and Biology of toxoplasma

According to Gustafson et al. (64), Nicolle & Manceaux (1908, 1909) describe the toxoplasma as follows: »... as crescentic, ovoid, or rounded; 4—7 microns long and 2—4 microns wide; occurring intracellularly or extracellularly, singly, in pairs, or in clusters; heteropolar, both ends being somewhat pointed, but one more than the other. The nucleus is round, variable in position but usually nearer the blunt end, not vesicular, and not influencing the shape of the cell. Occasional specimens show extranuclear granules, variable in size and shape, which are not kinetoplasts. The organism is nonmotile and no flagellum nor vestige thereof is present. Division is by longitudinal bipartition. This basic description has been repeated in substance by many other authors.«

Pulvertaft et al. (132) describe a central karyosome in the cell nucleus. The same has been observed also by Mühlpfordt (114) with Giemsa's staining and by Westphal (175) with Carnoy's fixation and subsequent hematoxylin staining. However, this formation was not observed by Gustafson et al. in electron microscope studies of thin toxoplasma layers. These authors found a 10 m μ thick membrane around the nucleus which had previously been assumed to be membraneless (143). Inside the nuclear membrane there was a dense irregular layer. Clusters of identical density were present also in the nucleus itself, but without fixed positions or dimensions.

Holz (78) states that the toxoplasma has 6 chromosomes. They can be seen in Fig. 10 by Hirschlerowa et al. (74). The nuclear

picture seems to be greatly dependent on the method of investigation.

The cytoplasm is not surrounded by a cell wall (64, 80). The cytoplasma reveals vacuoles and siderophil granules (183). Gustafson et al. state: »Occasionally a more compact group of granules is seen in a similar juxtanuclear position (..). Bodies suggesting mitochondria occur commonly ...» Holz & Bringmann (19) describe rod formations spreading from the pointed part of the toxoplasma inwards towards the cytoplasm. (See also the chapter on the taxonomy of toxoplasma.) They are evidently the same formations as those Gustafson et al. term toxonemes.

They are 14 — 18 in number and 0.08 — 0.12 μ in diameter, and converge on the base of the pointed part of the toxoplasma. The last-mentioned authors hold that these filamentous structures are peculiar to the toxoplasma. Chatton & Blanc (25) had already reported an optically denser formation in the pointed part of the toxoplasma. Westphal (175) called it trombicula, Gustafson et al. conoid. The latter consider that the base of the conoid is open towards the adjoining cytoplasm. It is not clear whether the distal end of the conoid is perforated. As to the conoid's function, they say: »Among the several possibilities that can be suggested must be included the designation of the conoid as a mouth structure, a penetration device, or a vestige of protozoal structure such as cytopharynx.»

The reproduction of toxoplasma probably always occurs inside a host cell or at least in the presence of living cells (143). Giraud & Gaillard (57) assume, however, that »free» extracellular forms may occur. Hirschlerowa (74) considers that the occurrence of »free» toxoplasmata is due to the careless handling of peritoneal exudate. The general opinion is that the toxoplasma propagates by longitudinal division (64, 104, 114, 188). The pointed end is divided first. This may result in rosette formations (114, 132, 175). Hirschlerowa says, however, that division of the toxoplasma takes place by »ultimate transversal division which is quickly followed by torsion of the axis and this may give the impression of a longitudinal division.» Bringmann & Holz (18) also believed, as a result of their electron microscope investigations, that the toxoplasma may divide itself in a plane at right angles to the longitudinal axis.

However, in a later publication Holz (78) holds the view that division is in the longitudinal direction.

As to the occurrence of schizogony and kinetic organelles, reference is made to the chapter on the systematics of toxoplasma.

Toxoplasma multiplies in tissue cultures at different rates. Sometimes the toxoplasma can rapidly fill the whole host cell whose nucleus is pushed aside (114). Mouse macrophages seldom contain more than 16 toxoplasmata (188). If the host cell »explodes», free toxoplasmata will be found in the surrounding tissue fluid. The occurrence of free toxoplasmata is to some extent rhythmic in occurrence (26, 188). Their appearance is dependent e.g. on the size of the infection dose. Vischer & Suter (188) showed in macrophage cultures that a 3: 5 ratio for toxoplasmata: macrophages gave free toxoplasmata after 19 hours. But with a ratio of 1: 20 no increase whatever in the number of toxoplasmata could be seen after six days even.

It is fairly generally agreed that the toxoplasma is capable of locomotion (79, 114, 188). The kinetic mechanism, however, is unknown. Jettmar (88) distinguishes between six different types of locomotion: (1) gliding forward movement, (2) lurching forward movement, (3) sudden backward movement, (4) rhythmic rotation, (5) dancing movement of two attached toxoplasmata, and (6) rippling movements. He points out that it is only in optimal conditions that these movements can be observed but then so clearly as to leave little doubt that the object is a protozoon and not a yeast organism. Manwell & Drobeck (108) claim however, that movement of a unicellular organism need not imply that it is a protozoon. These authors observed three types of locomotion with toxoplasmata. Pulvertaft et al. (132) speak of »jet propulsion» (Jettmar's movement No. 3 ?). They emphasize the toxoplasma is actively absorbed by the host cells but that it can also itself actively invade them. Macrophages, for instance, seem always to be the active part (188). It is possible that only the actively absorbed toxoplasmata are capable of multiplication. This would make for a dualism in the occurrence of the toxoplasma. This is reported also by Mühlpfordt (114). His tissue cultures of skeletal muscle cells and cartilage cells from chick embryos showed two types of toxoplasma: large toxoplasmata in the host cells showing

signs of degeneration, and small toxoplasmata that did not seem to affect the surrounding host cell. The vacuoles in the affected host cells may possibly be the result of the action of toxic products from the toxoplasma [Weinman & Klatschenko's (169) toxotoxin?] These products would not be formed by the small toxoplasmata which multiply rapidly and whose metabolism is adjusted for multiplication. It is possible that the latter type of toxoplasmata may give rise to the so-called pseudocysts which may be situated in totally reaction-free surroundings. It is possible that toxoplasmata can actively invade or be actively absorbed by the host cell only at a certain developmental stage of the host cell's mitotic division. Sometimes the mitotic division is then completed, sometimes arrested.

Several researchers have in certain cases observed a protoplasmic bridge between the host cell and the pointed end of the toxoplasma (74, 132, 175) and between one toxoplasma and another (88). Westphal (175) suggests that the protoplasmic bridge possibly may help the toxoplasma into the host cell. Whether this bridge attaches itself to the fixed point of invasion assumed by Pulvertaft et al. (132) is not clear.

Large toxoplasmata, up to 10 μ are sometimes seen in peritoneal exudate of mice without any apparent reason for their presence. The present author has failed to develop these toxoplasmata experimentally. It seems as if they were less adapted for a dye test.

Tissue cultures and patho-anatomical studies have shown that toxoplasma is highly non-specific to cells (104, 114, 132, 188). Cross & Anigstein (33) claim that the toxoplasma can only multiply in reticulo-endothelial tissue, but they make the concept of reticulo- endothelial tissue very extensive.

Taxonomy of Toxoplasma

It is unclear where the toxoplasma should be placed systematically. Incidentally, it is surprising that the biology and especially systematics of the toxoplasma should have attracted so little interest in the work expended on the toxoplasma problem in general. This is regrettable. If the position of the toxoplasma in nature were determined it would doubtless considerably facilitate the stu-

dy of its epidemiology. In any case, study of the systematics provides increased knowledge of the infection routes of toxoplasmosis.

Nicolle & Manceaux (116) first called the toxoplasma *Leishmania gondii*. However, as it had no blepharoblast they referred it to a new family, *Toxoplasmae*. It was then quite natural to classify the toxoplasma as a Sporozoa in order to adhere to the principal classification of protozoology, that based on morphological grounds. Schizogony, often observed in Sporozoa, was found by Splendore (154) in the strain of toxoplasma he isolated. Subsequently the occurrence of schizogony has been denied in several publications (84). Westphal (175), however, in a recently published work maintains that schizogony definitely can occur in toxoplasma. Opinions on this point, important from the classification point of view, thus differ. Another important morphological characteristic on which there is a divergence of opinions is the existence of exterior locomotive organs. Nicolle & Manceaux saw no flagella formation, but Splendore saw two flagellae, one at each end of the toxoplasma. Cross (32) later described flagella formations. He refers toxoplasma to the class Mastigophora. On a single occasion Piekarski too observed flagellae in toxoplasma. The general view has been, however, that toxoplasma has no external locomotive organs whatever. In the publication cited. Westphal described flagella formations in toxoplasma. He considered it definite that in certain conditions the toxoplasma has some kind of exterior organella protruding from the pointed end, the so-called trombicula. Whether a filapodium or flagella is involved has still to be made clear. But, as Westphal says, the difference between filapodium and flagella is one more of degree than of principle. That toxoplasma has no blepharoblast need not be interpreted to mean that the organella in question must be a filapodium as e.g. Werbitski (175) described certain trypanosomes without or with a detached blepharoblast.

Jettmar (88) holds that a toxoplasma with a 0.1 μ thick filament at its posterior end can attach itself e.g. to leucocytes. It is with this filmaent that the toxoplasma can make its sudden backward movements (Movement No. 3). At the anterior end of the toxoplasma Jettmar observed formations similar to flagellae. They seem to be shorter than the toxoplasma and produce rippling move-

ments (Movement No. 6). Jettmar's investigation may perhaps explain the two flagellae Splendore observed.

Hirschlerowa & Kozar (74) also reported flagella-like protoplasmic projections, shorter than the toxoplasma. However, they are only considered to occur in immature forms of toxoplasma. When toxoplasmata are expelled from a ruptured leucocyte they may remain attached to it by a protoplasmic bridge. The last-mentioned authors assume that it is just these toxoplasma forms which were described by Piekarski as being similar to spermatozoa.

Holz (79) says that especially the younger toxoplasmata move with boring-rotating movements at a temperature of 38—40° C. No kinetic organelles could be seen by ordinary microscope. With an electron microscope he observed flagella-like kinetic organs, resembling mucous filaments. He showed by a special method that these organs started in the trombicula. The base of these mucous filaments was optically denser. The mucous filaments run towards and over the nucleus but leave the rounder part of the toxoplasma free. Holz assumes that these organelles can contribute to or directly enable the movements of the toxoplasma, as they do with Myonemes, Ciliata, and certain Gregarina.

There is, then, no absolute agreement as to the occurrence of schizogony and filaments in toxoplasma. It is possible that the various toxoplasma strains differ and that this is partly responsible for the divergences. The environment and the different developmental stage of one and the same strain is probably decisive; Westphal (175) saw filaments only in a certain kind of toxoplasma preparation.

Hence toxoplasma cannot be classified on morphological grounds alone. We can perhaps agree with Jakobs (84): »We get little aid in this regard from a study of its morphology».

From a wider biological viewpoint it seems less suitable to refer toxoplasma to the class Sporozoa. The sporozoa are host-specific as a rule, whereas toxoplasma is extremely non-specific to host and cell. The sporozoa generally show sexual developmental stages in arthropods. All attempts to demonstrate sexual stages of toxoplasma in insects have failed. And even attempts to show that

the toxoplasma can be transferred by arthropods have generally failed.

Otto (121) refers toxoplasma to the *Haemosporidia* order of the Sporozoa class. He ranks toxoplasma with the *Plasmodia*. His theoretical speculations are not convincing. In addition, Otto refers the trypanosomes to the class Sporozoa, which is confusing.

Craing & Faust (31) refer toxoplasma to *Sarcosporida*. Manwell & Drobeck (108) consider that there is a close biological relationship between sarcosporidia and the toxoplasma. Kean & Grocott (92), however, find this a less appropriate classification, because the systematic position of sarcosporidia is unclear. It might be advisable in future attempts at classification to work parallelly with toxoplasma and sarcosporidia.

Manwell & Drobeck (108) say: »There was no evidence of flagella or the usual accessory structures seen in flagellate protozoa, either in living or stained organisms. Behavior was strongly like that of Sarcocystis spores. This fact with other evidence points strongly to a close biological relationship of the two genera. There seems much reason to question the general practice of including either with the protozoa.» Manwell & Drobeck are probably the only authors at present who do not consider toxoplasma a protozoon. On the other hand, Westphal and Jettmar find that the toxoplasma cannot be a yeast.

In an investigation into the systematics of toxoplasma, Westphal comes to the conclusion that toxoplasma belongs to the family *Trypanosomidae* of the class Mastigophora, and must be considered a *Leishmania* without blepharoblast. In that case toxoplasma would be the highest developed trypanosome that has adapted itself to the vertebrates.

Jettmar also holds that toxoplasma belongs to the class Mastigophora and is closest to the *Leishmania*. He assumes that a part of the developmental cycle of toxoplasma takes place outside the host cells.

II. SEROLOGIC DIAGNOSIS AND SEROLOGY

Serologic Diagnosis

Nicolau & Ravelo (115) described the first complement fixation reaction for toxoplasma in 1937 (CF), since when several different serologic methods for the determination of toxoplasma antibodies have been advanced. The bulk of these methods have been worked out since 1939 when toxoplasma was conclusively shown to be of importance to medicine.

In 1937, Sabin & Olitsky (143) described a »mice protection» test. The same authors also worked out the semi-quantitative neutralization test with rabbit (NT). This method, improved by Sabin & Ruchman (144), assumed fundamental importance for examining immunity in connection with toxoplasmosis. However, the method is both slow and expensive, and is today of historical interest only. The »pock count» method with eggs is essentially a more economic variant of the rabbit neutralization method. It was introduced by Alm (5) and Macdonald (110) but is hardly employed any longer.

In 1949, Sabin & Feldman (141) described a dye reaction in connection with toxoplasma. This, now called the Sabin-Feldman dye test (DT), has after minor modifications become the most generally employed serologic method for toxoplasma studies.

In 1949, Frenkel (47) reported on a skin test he had developed. The method is similar to that of the tuberculin test and is analogously called the toxoplasmin test.

Westphal & Palm (178) describe a hamster test with special »Einschlusskörperchen» in the hamster's liver ten weeks after an

intraperitoneal inoculation of toxoplasma suspension. The animals simultaneously become serologically toxoplasma-positive, which is taken to imply that the animals had had a toxoplasma infection. The reliability of this method, in other words the specificity of the »Einschlusskörperschen», has not yet been proved in the present author's opinion.

The systematic use of animal passages to demonstrate toxoplasma e.g. in liquor and infected tissue has not as far as is known been applied to large materials by any one but Wildführ (183, 184, 185). His results are of interest in that he managed to show toxoplasma with his method in 45 of the 315 cases studied. Toxoplasma was isolated from the liquor in 39 cases, from enucleated eyes in 4, from the placenta in 1 case, and from a section of a blood vessel in 1 case (a patient with Endangitis obliterans). The number of successful isolations was surprisingly high even for a selected material.

Of the various methods, the complement fixation reaction, dye test and toxoplasmin test are the most commonly applied. The first two have been employed in the present investigation, for which reason they are discussed in greater detail in the next chapter. As the toxoplasmin test has been specially recommended for epidemiologic studies this test too is discussed in detail and compared with the other two in a later chapter. The toxoplasmin test has not however been used in the present investigation.

Complement Fixation Tests

Nicolau & Ravelo (115) were probably the first to prepare (in 1937) an antigen for complement fixation tests in toxoplasmosis. In 1942, Sabin & Warren (145) made an antigen for complement fixation tests in toxoplasmosis. They found that peritoneal exudate from mice intraperitoneally infected with toxoplasma was, if used fresh, anticomplementary. Peritoneal exudate treated with methyl alcohol or ethyl alcohol gave haemolyzing and therefore unusable antigen. Antigen made from infected mouse brain did not fix the complement in the presence of toxoplasma antibodies or it reacted non-specifically; in other words, uninfected mouse brain fixed

complement in the presence of normal serum or in physiological saline solution.

Rabbits infected intracerebrally with 0.5 ml of a 10 per cent suspension of toxoplasma-containing mouse brain, died 4—8 days later. The brain of the rabbit was ground in a mortar and physiological saline solution added so as to make a 10 per cent suspension. The suspension was frozen in solid CO_2 and alcohol, and then melted. This was repeated 5 times. The suspension was centrifuged for 30 min. at 3.000 r.p.m. The supernatant was employed as antigen.

This antigen fixed the complement in the presence of toxoplasma-immune serum from immunized monkeys. 1—4 weeks after the monkeys had been infected with toxoplasma the complement fixation test gave a positive result. The titre values varied between 1/4—1/90 (initial serum dilution). The complement fixation returned to negative sometimes within 2 months even. If the monkey serum was stored deep-frozen for a longer period nonspecific reactions were found in the complement fixation test. This applied to both the normal and the immune sera.

Four rabbits immune to toxoplasma failed to give complement fixation in the presence of the above antigen. Four dogs and three cats examined before and after infection with toxoplasma gave a negative reaction throughout. In 20 persons with toxoplasma-neutralizing antibodies no complement fixing antibodies could be shown. None of these 20 showed signs of fresh or active toxoplasmosis. Warren & Sabin assume that the reason for the discrepancy between neutralizing and complement-fixing antibodies may be:

1. complement-binding antibodies disappear faster than the neutralizing,
2. inactive toxoplasmosis in the 20 persons examined,
3. the antigen was weak.

Warren & Russ (165) found that antigens made from infected or uninfected rabbit brain sometimes give a non-specific reaction. They made an antigen of the chorio-allantoic membrane of chick's egg.

The method was as follows. A 10 per cent suspension of toxoplasma-infected mouse brain was injected into eggs. After 7 days the egg membranes were collected and pooled. All the membranes had macroscopically observable lesions. The membranes were ground in physiological saline solution to a 10 per cent suspension (pH 7.4). The suspension was deep-frozen and melted 3 times, and then cenrifuged for 15 min. at 3,500 r.p.m. Normal 16-day eggs were treated similarly to obtain control antigen.

The complement-fixing material in this antigen was a specific, soluble substance, for it could be separated from the intact organism. The highest concentration of this substance was found in the embryo and the allantoic membrane. The antigen was relatively stable and could be stored without

undergoing any change for a minimum of 3 months at —20° C, or lyophilized. Sera stored at —20° C for periods ranging from a few days to 3 years were tested with this antigen. The highest titre obtained in these tests was 1/128.

In four sera from normal persons, both the infected and the control antigen gave complement fixation. The same applied to 3 sera out of 14 with a positive Wasserman reaction. All the normal sera with a non-specific reaction derived from persons vaccinated with vaccines prepared in eggs. A generally known fact is that positive Wasserman sera can give a non-specific reaction in other serologic tests. All of 47 persons with various eye diseases gave a negative reaction with Warren & Russ antigen.

Sabin (138) points out that the method employed by Warren and himself in their first investigation with antigen made from rabbit brain was unsatisfactory in that the sera were inactivated only at +56° C and not at +60° C, the antigen-antibody-complement mixture was incubated for only 30 min. at +37° C, and the potency of the antigen was unknown.

As e.g. Kidd & Friedewald (94) and Haven et al. (69) have shown that a non-specific complement-fixing factor can be removed from normal and from infected tissue by centrifuging at high speeds, it was natural to try to remove by centrifuging the non-specific component of toxoplasma antigen made from chorioallantoic membranes. Sabin says: »Centrifuging 13,000 r.p.m. for an hour was capable of improving the specificity of the toxoplasma antigen prepared from infected chorioallantoic membranes without changing its potency. With this improved antigen, even a serum titer of 1:2 to 1:4 was significant and indicative of the presence of specific toxoplasmic component fixing antibodies.»

In the same work Sabin compares antigens made from infected mouse brain and from both the supernatant and the centrifugate of peritoneal exudate. Sabin prepared his antigen from centrifugate as follows. A 1:10 dilution of 0.20 ml of peritoneal exudate from mice injected intraperitoneally 4 days before was injected in the same way into mice of a bodyweight of 20 g. The peritoneal exudate was collected after 4—5 days. (He found the exudate to contain some 19 million extracellular toxoplasmas per ml.) The exudate was then centrifuged for 20 min. in an angle centrifuge at 2,000 r.p.m.

Antigens were prepared separately from the supernatant and the precipitate. It appeared that the cells plus the toxoplasmas accounted for approx. 1 per cent of the total volume. Enough physiological saline to make a 2.5 per cent solution was added to the sediment (1/40). Both antigens were frozen in solid CO_2 plus alcohol, and melted at +37° C. This was repeated 10 times. The antigens were centrifuged for 20 min. at. 2,000 r.p.m., then centrifuged at 13,000 r.p.m. for an hour. Merthiolate up to a final concentration of 1:10,000 was added to the ready antigens.

The antigen from the peritoneal exudate of mice was anticomplementary, and this effect could not be eliminated even by centrifuging for another hour at 13,000 r.p.m.

By diluting the supernatant 1:16 and the antigen made of the sediment 1:4 the anticomplementary effect could be eliminated. But, says Sabin: »It should be noted however, that even after centrifuging 13,000 r.p.m. for 2 hours the preparations from mousebrains or peritoneal exude (the latter used in a conc. devoid of anticomplementary activity) were capable of fixing complement »non-specifically» with those few human sera which reacted with the normal chorioallantoic membrane antigen before but not after centrifuging at 13,000 r.p.m. for 1 hr.»

Sabin's experiments reveal that there is as much specific complement-fixing antigen in mouse brain as in the antigen from chorioallantoic membrane. Egg antigen, however, is more economic to use. Both the part containing toxoplasma and the cell-free part of the peritoneal exudate from mice contain more antigen per ml than egg antigen. In diluting the antigen to avoid the anticomplementary effect of peritoneal exudate antigens care must be taken to ensure that the antigen quantity required (4 units) is contained in the final dilution. In addition, there is no good control antigen available. As, moreover, the »high speed» centrifuging does not remove the non-specific component, Sabin recommends for routine use the antigen produced by Warren & Russ. But he makes the reservation that the non-specifically reacting component must have been eliminated by »high speed» centrifuging.

The antigen must be titrated with 16—32 units of antibodies to ensure that the final antigen contains 4 units per 0.25 ml.

The sera to be investigated must be diluted 1:2 and inactivated for 20 min. at +60° C. For dilutions over 1:16 the pipette must be changed frequently. The titres quoted refer to the original dilution of the serum, i.e. before the complement and antigen have been added. The sera giving a haemolysis of not more than 50 per cent are considered to have a positive reaction if they at the same time fail to react with the control antigen. The lowest serum concentration employed by Sabin in the control antigen was usually 1:2.

Sabin states: »Using the improved antigen not a single instance was encountered in which a positive complement-fixing test (titer 1/2 or more) was not associated with the presence of neutralizing antibodies for toxoplasma.»

Complement-fixing antibodies may persist for up to 6 years, or even longer. Positive complement fixation reaction can sometimes be taken as an indication of active toxoplasmosis. A low dye-test and negative or weakly positive complement-fixing reaction are

considered by Sabin as indications of past toxoplasma infection.

The antigen produced by Macdonald (110) must be considered a modification of that by Warren & Russ. He adds 1.5 ml of phosphate buffer solution per membrane (M/250, pH 7.4). The freezing and melting process is here repeated 3 times. Macdonald says: »Antigen prepared in this way may be anticomplementary in low dilutions, but this has not interfered with the performance of actual tests.» He used the antigen in a 1: 18 dilution as this strength continued to give maximal titres and was not anticomplementary. Fixation of complement with antigen antibody at +4° C overnight gave higher titres than fixation at +37° C.

Westphal (174) reports on a new method of preparing antigen from the peritoneal exudate of mice infected with toxoplasma. Healthy, large white mice were employed. The exudate was washed out of the peritoneal cavity with 3.8 per cent sodium citrate solution. The exudate was then treated in 7 different, quite complicated work phases. The protoplasm of the leucocytes and histiocytes was destroyed by ultrasonic treatment. Their positively-charged nuclei reacted with the negative citrate molecules, and under the influence of the ultrasonic waves they were baked together into a foaming mass. The toxoplasmata recovered by further manipulations were suspended in physiological saline 0.5 ml per mouse. The solution was then subjected to ultrasonic treatment for 20 min. An equal quantity of 1 per cent phenol solution was added to the resultant solution. The solution was kept overnight at +56° C. This treatment eliminated non-specifically reacting proteins to a considerable degree. The antigen obtained could usually be diluted further with physiological saline solution. The antigen had very good keeping qualities. No control antigen was used.

It could be shown experimentally that *Trypanosoma lewisi* and *T. equinum* gave non-specific reactions with this antigen. Otherwise the results with this antigen agreed well with those obtained by the Sabin-Feldman dye test.

Westphal & Bauer (176) used the method described above to prepare an antigen from the peritoneal exudate of guinea-pigs. They employed a toxoplasma strain adapted by Bauer for guinea-pigs. It turned out that 3 per cent of 266 sera reacted unspecifically with the mouse antigen but not with the guinea-pig antigen. Otherwise the modes of reaction of the two antigens showed close agreement. As it is possible that the small amount of mouse protein present in the antigen made from mice can react with the guinea-pig complement Westphal found it preferrable to prepare the antigen from guinea-pigs. The antigen made by Westphal has been produced on a factory scale and has been on the market for a couple of years.*)

Steen & Kåss (155) assume that the anticomplementary activity of the previously investigated antigens made from the peritoneal exudate of mice may have been due to the addition of heparin. They produced antigen in the following way. White mice were inoculated intraperitoneally with the RH

*) Messrs. Hoechst Leverkusen. The firm has now discontinued production.

strain. Four days after the inoculation the peritoneal exudate was collected without adding anything that might have prevented fibrin formation. The exudate was centrifuged for 30 min. at 3,000 r.p.m. and the mouse protein-containing supernatant was discarded. A volume of saline equal to that of the discarded supernatant was added to the precipitate. The suspension was frozen and melted rapidly 5 to 10 times. The resulting antigen was clear and not anticomplementary. It gave 100 per cent complement fixation with serum known to be positive in a dilution as high as 1:8 — 1:16.

The latest experiment made to prepare antigen from »pure» toxoplasma was that published by Behrens & Geissler (14). They transferred toxoplasmata and cells from peritoneal exudate via acetone to petroleum ether and carbon tetrachloride. This separated the toxoplasmata and the cells. The antigen has not yet been tried out.

In one of their experiments Warren & Cutchins (34) used an antigen treated for 1 hour with sonic vibrator and then centrifuged for 30 min. at 10,000 r.p.m.

The Present Complement Fixation Studies with Toxoplasma Antigen

The present author started his experimental studies in 1951 in an attempt to prepare an inexpensive and useable antigen for complement fixation experiments with toxoplasma. No serologic or parasitologic investigations had then been carried out in the country.*)

It would doubtless have served the purpose best to use peritoneal exudate as the starting material for the preparation of the antigen as mice had to be employed in any case to keep the toxoplasma strain alive.

As was shown by Sabin (138), although the peritoneal exudate of mice is rich in soluble antigen unfortunately a non-specific, complement-fixing component cannot be eliminated by high speed centrifuging. This led the present author to study other methods of eliminating the anticomplementary component. An antigen made from the spleens of healthy mice was used as the control antigen for all the experiments with the antigen made from peri-

*) An investigation concerning the occourrence of toxoplasmosis in pregnant women with miscarriage and premature births and various other complications had been carried out by Zilliacus & Wist (195). The serologic determinations for their work were made by Gard in Stockolm.

toneal exudate (47). Parallel with these was employed an antigen made according to Warren & Russ (165) from the chorio-allantoic membranes of embryonated eggs, different only in that the chorio-allantoic membranes were infected with a 10 per cent suspension of toxoplasma peritoneal exudate from mice infected 3—4 days previously with 0.2 ml of a similar suspension. Streptomycin, 200 γ per ml, and penicillin, 1 mg per ml, were added to the toxoplasma suspension.

Complement titration in the presence of the different antigens gave the following results:

Table 1

Complement titration in the presence of different antigens

Antigen, see text	Dilution of complement								
	1/10	1/12.6	1/15.6	1/20	1/25	1/31.6	1/40	1/50	1/63
Egg antigen No. A	100*	100	100	100	100	75	50	0	0
Antigen No. A diluted 1:2	100	100	100	100	100	100	50	0	0
Egg control antigen	100	100	100	100	100	75	50	0	0
Peritoneal exudate antigen No. B	100	100	100	50	0	0	0	0	0
Antigen No. B treated with sonic vibrator	100	50	50	0	0	0	0	0	0
Control without antigen	100	100	100	100	100	100	100	50	0

*) 100 indicates complete haemolysis of red blood cells in the CF test.
0 no haemolysis.

With antigen No. A, a known immune serum gave a titre of 1:128 without giving any complement fixation with control antigen , antigen No. A diluted 1:2 gave the same titre. A third experiment showed that a 1:16 dilution of antigen No. A had no effect on the complement titration but lowered the complement fixation titre of a known immune serum from 1/1,1024 to 1/8. Later on each new batch of antigen was titrated against a known immune serum, and the antigen was then used in the dilution that

gave the highest complement fixation with this immune serum. The control antigen was similarly diluted.

A 1:1 dilution of mouse peritoneal exudate was employed as antigen and treated with a sonic vibrator (Raytheon 9 KC 50 W Magnetostriction Oscillator Model S-102 A). In accordance with the instructions, the frequency control was turned to the optimal position and the voltmetre needle set at 150 V. Penttinen (124), among others, has shown that sonic vibration increases the haemagglutination titre of various virus preparations. As is shown in Table 1, the anticomplementary effect of the antigen is increased by sonic treatment. This was corroborated by later experiments. At the same time the antigen was less potent than before the treatment. A potent egg antigen was also treated by vibrator and high speed centrifuging. The results are given in Table 2.

Table 2.

Complement titration in the presence of egg antigen

Antigen, see text	Dilution of complement								
	1/10	1/12.6	1/15.6	1/20	1/25	1/31.6	1/40	1/50	1/63
No. 1	100	100	100	100	100	100	100	50	50
No. 2	100	100	100	100	100	75	50	0	0
No. 3	100	100	100	100	100	100	50	50	50
No. 4	100	100	100	100	100	75	50	50	50
No. 5	100	100	100	100	75	50	50	0	0
No. 6	100	100	100	100	100	100	50	50	0
No. 7	100	100	100	100	100	100	75	50	0
No. 8	100	100	100	100	100	100	100	50	0
No. 9	100	100	50	50	0	0	0	0	0
No. 10	100	100	100	75	75	50	0	0	0
No. 11	100	100	100	100	100	100	100	100	50

*) 100 indicates complete haemolysis of red blood cells in the test, 0 no haemolysis.

A toxoplasma antigen and a control antigen prepared from embryonated eggs (Egg Antigen) according to Warren & Russ (see p. 21), were used for the experiment reported on in Table 2. However, the antigen was a 20 per cent antigen.

The antigen was further treated as follows:

No. 1 Treated with sonic vibrator for 5 min. (see above) and then centrifuged in Spinco high speed centrifuge at 38,200 g. for 1 hour.
No. 2 The control antigen treated as Antigen No. 1.
No. 3 Treated with sonic vibrator for 10 min. and not centrifuged.
No. 4 The control antigen treated as Antigen No. 3.
No. 5 Treated with sonic vibrator for 10 min. and centrifuged as Antigen No. 1.
No. 6 The control antigen treated as Antigen No. 5.
N:o 7 Antigen No. 5 diluted 1: 2.
No. 8 Antigen No. 6 diluted 1: 2.
No. 9 The original antigen untreated.
N:o 10 The original control antigen untreated.
No. 11 The antigen only high speed centrifuged.

Table 2 shows that vibrator treatment increases the anticomplementary qualities of the antigen, as was the case with antigen prepared from the peritioneal exudate of mice. High speed centrifuging alone is better than sonic vibrator treatment plus high speed centrifuging. It is possible that prolonged vibrator treatment (another frequency?), which was not tried here but which Warren & Cutchins (34) used, can eliminate the non-specific and anticomplementary effect. In any case, sonic treatment is better than no treatment at all where egg antigen is concerned. That the anticomplementary effect can be reduced by dilution is evident. However, this has its disadvantages, as Sabin showed with antigen from the peritoneal exudate of mice.

Antigens No. 1 and No. 3 (No. 9 was not titrated) gave identical titres with identical immune serum. No. 5 and No. 7 gave a titre lower by one tube.

Oker-Blom (118) has shown that certain virus suspensions can be purified by Bentonite** to improve them for use as antigens. Bentonite eliminates from the suspension mainly lipoproteins which, as is known, may act anticomplementarily. A complement titration in the presence of an egg antigen treated with Bentonite (Bentonite final conc. 1.2 per cent) revealed that the anticomple-

**) Bentonite = Montmorillionite.

mentary effect had been completely eliminated but the specific titre of the antigen had simultaneously fallen by three tubes.

A comparison between antigen prepared from the centrifugate of toxoplasma peritoneal exudate from mice, produced by adding to the centrifugate a volume of physiological saline solution equal to the discarded supernatant exudate solution, and an egg antigen prepared according to Warren & Russ (165) but centrifuged at high speed, gave the following results:

Table 3

Complement titration in the presence of antigens

	Dilution of complement					
Antigen	1/15.6	1/20	1/25	1/31.6	1/40	1/50
Egg antigen	100*	100	100	100	100	50
Egg control antigen	100	100	100	100	100	50
Mouse antigen 1:1	100	50	0	0	0	0
Mouse antigen 1:4	100	100	100	50	25	0

*) 100 indicates complete haemolysis of red blood cells in the CF test.
0 no haemolysis.

Table 4

Complement titration of an immune serum with different antigens

	Dilution of immune serum							
Antigen	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Egg antigen	0*	0	0	0	0	0	0	100
Egg control antigen	100	100	100	100	100	100	100	100
Mouse antigen 1:1	0	0	0	0	0	0	0	25
Mouse antigen 1:4	0	0	25	100	100	100	100	100

*) 100 indicates complete haemolysis of red blood cells in the CF test.
0 no haemolysis.

The table reveals that in antigen content the mouse antigen is as potent as the egg antigen but considerably more anticomplementary. This undesired side effect can of course be eliminated by dilution, as Table 4 shows. But the potency of the antigen is then considerably reduced.

The above experiments led the present author to use in his routine investigations, in spite of the higher cost involved, an antigen prepared in the following way.

THE PRESENT AUTHOR'S METHOD OF PREPARING ANTIGEN

(1) Embroynated 10-day old eggs were inoculated on »dropped chorioallantoic membrane» with 0.2 ml of a toxoplasma suspension according to a method indicated for virus technique.

(2) The above toxoplasma suspension was obtained from the pooled peritoneal exudate of mice inoculated 3—4 days previously with a similar toxoplasma suspension. The peritoneal exudate was centrifuged and the centrifugate diluted 10 times. To this was added 1 mg. of penicillin and 200 γ of streptomycin per ml.

(3) The inoculated eggs were kept in an egg incubator for 5 days.

(4) The parts of the chorio-allantonic membranes affected by toxoplasma were collected.

(5) The egg membranes were deep-frozen (—20° C) and melted (+37° C) 4—5 times.

(6) The membranes were weighed and a 20 per cent suspension was made in physiological saline solution.

(7) The suspension was ground for 3 min. in a laboratory mixer.

(8) The suspension was centrifuged for 30 min. at 2,000 r.p.m. and the supernatant collected: merthiolate in a final concentration of 1:10.000 was added.

(9) The fluid in Item (8) contained the soluble toxoplasma antigen but was anticomplementary to a varying degree and usually reddish (dissolved chick blood cells). If the antigen at this stage was allowed to stand at —20° C for 3—6 days a precipitate formed which had absorbed the red colour to a considerable degree. Hence the final centrifuging gave a more colourless antigen without any reduction of potency.

(10) The antigen was finally centrifuged for 1 hour at 38.200 g in a Spinco centrifuge.

(11) The resulting antigen had good keeping qualities and a high antigen content and was at the same time fairly slightly anticomplementary only.

The complement fixation technique was that simultaneously applied for other investigations in the laboratory. It is described by Penttinen & Halonen (125), largely as follows:

The total volume of reagents in each tube in titrations and controls was the same, 1.0 ml. The volume of each reagent pipetted into the tubes was

always the same, 0.2 ml. As a diluent a Mg++ containing 0.9 per cent sodium chloride solution was used throuhout.

Hemolytic amboceptor. The amboceptor used in our laboratories for Wassermann tests was titrated with complement diluted 1/15 in dilution series 1/1000, 1/2000, 1/3000, 1/4000, 1/6000, 1/8000 and 1/10,000. The dilution of amboceptor in complement titration and in the test proper was twice the last dilution showing complete hemolysis.

Complement. Pooled guinea-pig complement was titrated in the presence of antigen with a dilution factor of 1.26, beginning at a dilution of 1/10. The dilution of complement used in the serum and antigen controls was the last one showing complete hemolysis in complement titration, and in the test proper 1.26 -fold dilution.

Cells. 2.5 per cent washed sheep cells were used in all titrations.

Sera. The sera were inactivated and stored at —20° C until used or titrated the same day that they arrived at the laboratory. The dilution factor was 2 in the titrations and the first dilution 1/2.

The test proper. 0.2 ml of serum dilution, of antigen, and of complement in proper dilutions were incubated at +37° C for 45 minutes. Fifteen minutes before the addition of hemolytic system, equal amounts of cells and amboceptor dilution were mixed. To each tube 0.4 ml of sensitized cells was added and the incubation continued until the serum and the controls showed complete hemolysis. The last serum dilution showing fifty per cent fixation after an over night storage of the tests in a refrigerator at +5° C was considered the titre of the serum.

Controls. In all series, controls of the hemolytic system, the antigen and the serum were included, the dilution of the antigen being the same as in the test proper and the dilution of the serum 1/2. To each series a titration of known positive serum was added as a further control. Some variation in the titre of the same serum occurred in the different series but more than 1 tube up or down was not recorded.

DYE TEST (DT)

In September 1948, Sabin & Feldman (141) reported a new staining phenomenon in connection with toxoplasmosis, later developed by them into the so-called Dye Test (DT). This method of determining toxoplasma antibodies rapidly came to replace the neutralization test with rabbits and embryonated chick eggs. It was first believed that the DT measured other antibodies than the NT and that the NT antibodies were thermolabile (75). Later on, however, Winsser & Makstenieks (187) showed that an accessory factor similar to that in DT (see below) was required in NT and

that it was only this accessory factor that was thermolabile. The last-mentioned authors consided DT and NT antibodies to be identical.

In the DT, toxoplasma-immune serum or ordinary serum acts in different dilutions at +37° C on toxoplasmata in the peritoneal exudate of mice. If a thermolabile serum component is present in sufficient quantities in this reaction — a component called by Sabin & Feldman the »accessory factor» (AF) — the toxoplasmata undergo a change as a result of which their cytoplasm fails to stain subsequently with alkaline (pH 11) methylene blue solution. But methylene blue solution stains toxoplasmata exposed to the normal serum. The toxoplasmata from the different serum dilutions are best observed under a phase-contrast microscope. 50 or 100 toxoplasmata per dilution are counted. If over 50 per cent of the toxoplasmata counted are unstained the serum dilution involved is considered positive. In this way toxoplasmata in rising serum dilutions are counted until the limit of 50 per cent unstained is no longer reached. The immediately preceding serum dilution is the titre of the serum concerned, indicated as »original» serum dilution.

Sabin et al. (140), in 1952, recommended a DT technique for routine use. It is this technique that the present author has followed.

The original Sabin & Feldman DT method, with minor modifications, has been applied by a number of investigators. Winsser (186) uses a method by which the sera are measured by the drop. Beverly & Beattie's (13 a) dye test method will be discussed later. Special interest from the general serological point of view is aroused by Avad's (7) method of using *Sarcocystis tenella* spores instead of toxoplasma in DT for toxoplasmosis and sarcocystis infection.

A number of factors affects DT. In spite of this the individual investigator usually gets consistent results. The titre of one and the same serum may vary somewhat in different DT titrations. In order to obtain fully comparable results with different sera from one and the same patient they must be tested in the same DT, as Frenkel pointed out. He says that the same serum titre, especially with different accessory factor sera, can vary up to three

steps. The present author has never observed a titre difference of so many tubes in his experiments.

It can also be pointed out that a single serum tested by different investigators has given fairly concurring results. This applies e.g. to the sera submitted by Beverly & Beattie (13 a) to Feldman for control. After 4 years of experience with DT the present author is inclined to agree with Wildführ (183) who claims that the test is not easy to carry out and that it requires expert knowledge and experience; it is also expensive.

In the following the problems connected with the various DT factors will be discussed individually.

The Patient's Serum:

According to Sabin et al. (140), coagulated blood or serum can be submitted to the laboratory. Inactivation is not necessary. These authors recommend a first dilution of 1/16 for routine DT.

Westphal & Mühlpfordt (177) state that the effect of normal serum on toxoplasma can be increased by letting the serum stand at room temperature for some time. A serum they examined and that had stood at room temperature for a prolonged period affected the toxoplasmata so that 85 per cent of them failed to stain in spite of the fact that his serum in fresh condition had been completely DT-negative. They indicate that the serum must have a certain degree of dispersity to be able to function blamelessly in DT and that this dispersity increases when serum ages. This change in the serum's colloidal structure, as it is termed by Westphal & Mühlpfordt (177), can be removed by inactivation for 30 min. at +56° C. The specific DT antibody, on the other hand, is thermostabile. According to some authors, the specific antibody disappears rapidly from whole blood but, it is believed, is better retained in inactivated serum (177, 183). They recommend, therefore, that inactivated serum be submitted to the laboratory. However, this is likely to be difficult in practice, in Finnish conditions at least.

Beverly & Beattie (13 a) believe that blood submitted by mail which is a few days in transit undergoes no appreciable change. According to Vivell (189), the shaking of the serum in the mail may change it, and the resulting effect on DT and CF antibodies may be different.

Own observations

The present author considers it advisable to inactivate the serum before DT to ensure that a »standardized» amount of accessory factor is present in every tube. It is possible that in this way non-specific reactions in low serum dilutions can be avoided. (See also below concerning the accessory factor.) Toxoplasma-immune sera retain their titre over a very long period if stored at —17—20° C. The present author kept a serum with a DT titre of 1/256 for over 2 years without its titre changing. However, sera with high titre values seem to drop more easily, by one or two tubes.

It may be mentioned that a serum with a titre of 1/16,386, which was sent by mail and was 4 days on the way, dropped to 1/4,096 while its CF titre remained at 1/32.

Accessory Factor (AF)

Sabin & Feldman (141) showed in their basic experiment that DT required a thermolabile serum component to supplement the reaction. They called this factor the accessory factor (AF). AF is present in fresh normal human serum. It is hardly found at all in mice and guinea-pigs, They found that a 40—50 per cent dilution of AF was enough to prevent the immunity phenomenon in more than 70 per cent of the toxoplasmata. It turned out that fresh serum from cow, sheep, dog, monkey, rabbit, rat and guinea-pig was able alone to change in varying degree the affinity of toxoplasma to methylene blue. This was considered to be due to a »normal, heatlabile antitoxoplasma factor». The authors failed in their attemps to show this factor in man and in white mice.

Sabin & Feldman consider that though AF resembles the ordinary complement (C') it differs from the latter in many respects, one instance being that the amount of AF required to give full haemolysis is greater than the amount of C'. They failed to restore the activity of a heat-treated AF serum with pure C'_1 and C'_2 preparations. However, this serum was active as ordinary C' in the lysis of red blood cells in complement fixation tests.

Sabin & Feldman found that the DT antibody bound with the toxoplasma also in the absence of AF. These toxoplasmata stained beautifully with methylene blue and were fully virulent for

white mice. But AF does not bind with toxoplasmata unless DT antibodies are present.

Westphal & Mühlpfordt (177) reported that a surplus of AF must be present to give good DT results. They consider that AF has relatively good keeping qualities and can be stored at 0° C for 8—10 days without spoiling. However, older AF contains a non-specific component, as does ordinary human serum. This can, as is the case with ordinary serum, naturally not be removed by inactivation for 30 min. at +56° C.

Roth (135) investigated AF for its components and found that C'_2, C'_3 and C'_4 were common to both the »ordinary» complement and to AF. C'_1, however, was not necessary to obtain an AF effect but the factor which in the yeast treatment of ordinary C'-sera was removed from it together with C'_3 was absolutely necessary for an AF effect.

The present author (60) has investigated AF and found that it probably consists of

$$\text{Properdin} + C'_2 + C'_3 + C'_4.$$

It seems that a major amount of properdin is required for a complete AF effect.

Properdin, first isolated by Pillemer et al. (131), is an euglobulin with its lowest solubility lying between pH 4.8—6.5. It constitutes approx. 0.03 per cent of the proteins of a given serum. In its pure form it tolerates warming up to 66° C for 30 min, but is destroyed in 5 min. at 100° C. Properdin is not a component of the blood clotting mechanism or of the haemolytic complement system. Properdin participates in bactericidal, virus-neutralizing and certain haemolytic reactions in the presence of complement and Mg^{++}. It is believed to be a factor in natural immunity.

Weigand & Gleim (167) found that AF serum control could suddenly be found to contain plenty of unstained toxoplasmata. They considered that the phenomenon occurred especially in peritoneal exudate containing an abundance of toxoplasmata. According to them, the cause of the phenomenon was the mice which in their case formed antibodies more rapidly than usual. The same AF serum could simultaneously react blamelessly with peritoneal

exudate from another mouse. They pointed out that DT could only be carried out by specially equipped laboratories.

Gard (52) states that it is difficult to find a suitable blood donor with a high AF content without at the same time changing the stainability of the toxoplasma. Wildführ (183) indicates that 17 per cent of the normal sera are suitable for use as AF sera.

Present observations

To start with the present author had the impression that the problem was the following: it was necessary to find a person with a suitable properdin concentration, high enough to ensure that the DT would function but not so high that the properdin itself would change the toxoplasmata to non-stainable with methylene blue. Later it turned out that a high properdin content even leaves the toxoplasmata apparently unaffected. If toxoplasma-immune serum is present at the same time toxoplasmolysis is accelerated when the properdin content is high. In this case no beautiful unstained toxoplasmata will be seen, only the deformed unstained remnants of toxoplasmata. Pure bovine properdin, kindly made available by Prof. Louis Pillemer, was employed in these experiments. The highest final concentration of properdin in these experiments was 30 units/ml; the lowest concentration that gave full effect with a known immune serum was 1.5 units/ml. With a good immune serum, the properdin content in different sera can be readily titrated.

The Dye

The dye recommended by Sabin & Feldman (141) for DT is methylene blue in an alkaline (pH 11.0) solution. An alkaline solution hastens the staining process considerably without otherwise affecting the reaction. The dye must be newly prepared from parent solutions (see p. 39). According to the present author's experience, prepared dye solution is usable up to the 6th—8th day. However, it is recommended that a newly prepared dye solution be allowed to stand for 1 hour before use.

Sabin & Feldman (141) emphasize that dyes other than methylene blue can be employed in DT. The dyes they investigated are of the following types: (a) dyes that do not stain the positive toxo-

plasmata but do stain the negative belong to the quinone-imine group and are all basic dyes. Of them, methylene blue, thionine and toluidine blue belong to the sub-group of thiazones. (b) dyes that stain neither DT-positive nor -negative; the group includes e.g. the disazo dyes congo red, brilliant vital red and trypan red. all of them acid dyes.

These two groups suggest that only basic dyes are effective in DT. This is doubtless far from true. The staining phenomenon in this case is hardly a simple acid-base reaction. Sabin & Feldman show also that the acid fluorescein derivative phloxine, a dye of the xanthene group, stains DT-positive but not DT-negative toxoplasmata.

Obivously, there is no consistency about the staining phenomenon and the tendency within the different dye groups suggests that auxochrome groups of a certain type are active in the staining phenomenon. It is possible that histochemical methods would provide a means of studying further the phenomenon involved in the immunochemical process in DT, a phenomenon which evidently leads to relative toxoplasmolysis. Three of the dyes investigated by Sabin & Feldman (141) might suggest relative toxoplasmolysis. The phloxine that Unna (164) employed for studies of chromolysis can be assumed to stain only split remnants of the cytoplasm. The Mg^{++} content bound to the surface (?) of the toxoplasma by the immunologic reaction may also affect the phloxine stainability which is known to be sensitive to alkali metals in very low concentrations even (30). Basic fuchsin, included e.g. in Schiff's reagent, is considered a practically specific indicator of free aldehyde groups. Hence it may be assumed that a splitting of the cytoplasm of the toxoplasma, a process which possibly occours in positive DT, leads to a number of free aldehyde groups both in the remnants of the cytoplasm left within the toxoplasma and their staining by the basic fuchsin. The positive toxplasmata therefore stain more strongly, the negative hardly at all.

It is slightly surprising that the German authors as a rule report low DT titre values. Westphal (173) investigated the matter and assumed that it was due to the quality of the dye employed. The methylene blue preparation he applied in routine use gave, on an

average, four times lower titre values than the methylene blue preparation used by Sabin & Feldman. This concurs well with the fact that the German titre values are usually four times lower than those reported by others.

Other groups of authors also have investigated the significance of the methylene blue preparations for the DT titre but failed to establish any difference. For instance, Beverley & Beattie (13 a) investigated six different preparations without finding a difference between them. On the other hand, it would be surprising if all the methylene blue preparations give identical results as the variation in their quality is immense. This applies particularly to the ratio the azure content bears to the period during which the dye is kept prepared.

The present author's experiments with the different methylene blue preparations gave the following results:

Table 5

Dye test with different methylene blue preparations

With a serum known to have the titer 1/1204 in routine DT.

The Dyes	Fresh dye solutions						7 day- old dye solutions					
	A	1/16	1/64	1/256	1/1204	1/4096	A	1/16	1/64	1/256	1/1024	1/4096
Metylenblau stand. »Bayer» Leverkusen. No. 550182	2	96	96	70	54	4	6	92	94	88	64	12
Methylene blue G. T. Gurr England 6666	2	74	82	49			8	92	68	44		
Methylene blue chloride H. D. Conn USA	4	88	84	64	24		6	92	72	60	64	28

Metylenblau BB. E. Merck. Darmstadt.	4	80	86	42		10	98	84	64	40
Metylenblau No. II E. Merck.	6	98	86	56	14	4	96	80	44	

With a serum known to have the titer 1/4 in routine DT and with the dyes listed above.

The dyes	Fresh dye solution				7 day- old dye solution			
	A	1/4	1/16	1/64	A	1/4	1/16	1/64
»Bayer»	2	70	12		6	60	32	
»Gurr»	2	34	8		8	68	24	
»Conn»	4	62	16		6	72	28	
»Merck BB»	4	58	36		10	60	24	
»Merck II»	6	58	12		4	56	20	

The figures indicate the per cent of unstained toxoplasmata in the respective dilution of the dye test.

Column A gives the per cent of unstained toxoplasmata in the »stainability control.»

As can be seen from Table 5, different titre values can be obtained with the different methylene blue preparations. For instance, a serum with a titre of 1/4 would doubtless have been classed as negative on the first test with the Gurr methylene blue preparation.

The experiment shows that methylene blue preparations tolerate fairly well storage at room temperature for 7 days. The two Merck preparations are perhaps poorest in this respect. The Gurr preparation actually improves with storage. The in the stainability control (column A) is higher throughout in the latter experiment. This may of course depend on the peritoneal exudate, although it was taken from evenaged mice inoculated in the same way as the exudate for the experiment with fresh dyes. AF was the same in both cases.

For routine use the present author employed the methylene blue preparation stand. »Bayer» Leverkusen, which seems comparable with Methylene blue Conn. A point of special interest is that dye

preparation in routine use by the present author is the same as that employed by Westphal & Mühlpfordt (177); they used Methylenblau stand. Behringwerke which it seems was later marketed as Methylenblau stand. »Bayer» Leverkusen. In spite of this the present author has several sera with high titre values, e.g. one with a titre of 1/65,386 from a patient with glandular toxoplasmosis (62). The factor finally responsible for the difference between the titre values reported by German and other authors is still unclear. Jacobs & Cook (85) assume that the titre differences are to be referred to the ratio of peritoneal exudate to accessory factor. They show that ratios of 1/2, 1/4 and sometimes 1/5 give no clearly readable results with known immune sera, whereas ratios 1/5—1/10 give definite positive results with the same immune sera.

Peritoneal Exudate

The phenomen described by Jacobs & Cook (85) is considered to be due to dissolved antigen present in the exudate itself; the antigen must be sufficiently diluted in AF serum. Wildführ (183) used the method of injecting 2.0 ml of physiological saline solution intraperitoneally into mice with toxoplasmic peritoneal exudate. This gave him the maximum amount of exudate from the mice. In routine work, from the experience of the present author, 0.4—1.0 ml of pure exudate is obtained. In other words, with Wildführ's method the ratio of exudate to AF serum will vary between 1/9 and 1/18. He used 0.05 ml of toxoplasmic suspension + 0.1 ml of AF serum. All the same, Wildführ reported low titre values.

For the present investigation the peritoneal exudate was always used as it was, with an addition of heparin (which is known to be anti-complementary) in a ratio of 1/4 with AF serum. In spite of thist the titre values obtained are high, as was pointed out. No final conclusion can be offered yet, therefore, as to the factors responsible for the titre differences of German and other authors.

Several authors, e.g. Beverley & Beattie (13 a), emphasize that only young mice are suitable for DT. Older mice are believed to be capable of developing DT antibodies and thus affecting the toxoplasmata in DT. The result is a low staining percentage in the stainability control. The same phenomenon is also observed in

young mice just before they die of toxoplasmic infection. Hence it is essential to use young mice and to take the peritoneal exudate on the 2nd to 3rd day. The upper limit of 5 days indicated by Beverley & Beattie seems too long. (The mice die on the 5th day.) From experiments with older mice it seemed as if more exudate formed in mice that had recently produced a litter or were pregnant.

Piekarski (129) claims that large mice yield stainable toxoplasmata more readily. He also claims that mice whose liver contains a certain developmental form of the parasite *Taenia taeniaeformis* yield a peritoneal exudate containing toxoplasmata a high percentage of which will not stain.

For no manifest reason whatever, a toxoplasma strain can suddenly turn out quite unusable for DT. This has been reported by Jacobs & Cook (85) also. The present author used the international RH strain and one of his own, isolated from the brain substance of case of congenital toxoplasmosis (61). This, the so-called Ko strain, was originally weakly virulent for mice, between the 45th and the 50th passage it suddenly became strongly virulent. In its biological behaviour it is now very similar to the RH strain. For instance, the »exhaustion phenomenon» described above and unsuitability for DT was observed in both strains though so far not simultaneously. One and the same immune serum gave indetical results with the two strains. But experiments by Wildführ & Hudemann (183) show that different strains can give different results. German investigators now use a so-called standard strain. It should be clear from the above that a standard strain even may sometimes be unusable in DT.

It is interesting to see that Lelong & Desmonts (103), who workeed with toxoplasma strains, were unable to carry out the DT. All the sera they investigated had a strong lytic effect on toxoplasma. As it was possible to carry out DT during a certain period (between 80 and 120 passages) with a toxoplasma strain they had isolated the conclusion they arrived at was that their toxoplasma strains were too sensitive to normal sera even.

The occurrence of the above »exhaustion phenomenon» is interesting. It can also be taken to suggest a kind of dualism in toxoplasma. The relationship between intracellular and the extracellu-

lar toxoplasmata varies from time to time and can be assumed to affect the suitability of the exudate for DT. Visher & Suter (188) descriebed an interesting phenomenon that they had observed in tissue culture of toxoplasma. Approx. 8 hours after the culture had been infected with toxoplasma, stained intracellular toxoplasmata were observable. The nuclei of the toxoplasmata were stained but their cytoplasm failed to stain with haematoxylin. Whether this has something to do with the above »exhaustion phenomenon» is unclear.

The reading of the test itself, as mentioned previously, is done by counting the number of unstained and stained toxoplasmata. The phase-contrast microscope is recommended for the purpose as it provides a means of ensuring that no unstained toxoplasmata espace unnoticed. The present author employed oil immersion; this is usually considered unnecessary, but it does enable reading in detail although it slows the work down. There is no doubt that DT involves a quantitatively balanced immunologic reaction. Against this background there may well be some justification for the assertion by Beverley & Beattie (13 a) that the number of toxoplasmata in the reaction is of importance to the titre value. They suggest 30,000 toxoplamata per ml as the standard limit. Sabin et al. (140) indicate, however, that the number of toxoplasmata makes no difference to the reaction. In the present work the method of the latter authors has been followed; the number of toxoplasmata in the reaction has thus not been counted.

SKIN TEST WITH TOXOPLASMIN

The skin test with toxoplasmin was first described by Frenkel (47). The principle is the same as for tuberculin. Frenkel employed toxoplasma-containing peritoneal exudate from mice infected 4—5 days previously with 0.2 ml of a 1:50 dilution of toxoplasmic peritoneal exudate. Heparin-containing peritoneal exudate was centrifuged for 20 min. at 2,000 r.p.m. The sediment was dissolved in a tenfold quantity of physiological saline solution and exposed to ultraviolet rays to kill the toxoplasmata. The suspensions were frozen and thawed 5-10 times. The toxoplasmin was em-

ployed in a dilution of 1: 1,000 with an addition of 0.25 per cent of phenol. A preparation similarly made from mouse spleen was used as the control. Mouse spleen is believed to contained very nearly the same cells as toxoplasmic peritoneal exudate, apart from the toxoplasmata themselves (47). The toxoplasmin and the control solution can be tried out on experimentally infected guinea-pigs.

Feldman & Sabin (43) used toxoplasmin made of toxoplasma-infected chorioallantoic membranes.

The antigen prepared by Westphal (174) for complement fixation reaction has also been tested as toxoplasmin.

In the skin test 0.1 ml of the toxoplasmin is injected intracutaneously e.g. into the forearm. More than one dilution of toxoplasmin is not recommended as the correlation between the content of toxoplasma antibodies in the blood and the sensitivity of the skin to toxoplasmin is apparently not particularly good (43). Readings are taken after 24 and 48 hours. The reaction is considered positive if the erythema and induration are over 10 mm in diameter. The skin test is not suitable for children under 2 years or for the aged (160).

Kozar (96) showed that the size of the skin reaction in strongly positive persons increases between 24 and 48 hours after the inoculation. If the reaction is positive after 24 hours but remains under 10 mm at the end of 48 hours he considers it non-specific.

Thalhammer (163) suggested that the allergometric method worked out by Gröer for tuberculin reactions be employed in ST. He hoped to distinguish in this way between potentially seriously and innocuously infected persons. Vivell (190) found this method to be based on uncertain grounds. Kozar (96) examined 179 persons with the allergometric method and found that 100 of them were positive; 39 of the positive had a pleoaesthetic reaction. In a more normal study material Thalhammer had 11 pleoaestheticians among the 100 examined. On clinical grounds Kozar's material was considered to include several patients with active toxoplasmosis. Kozar believes, therefore, that the allergometric method may possible be useable. Hewover, he points out that sometimes it is difficult to assess the skin reaction and that, used as a supplement to DT and CF, the method perhaps gives a better idea of the patient's immune-biological condition.

By way of summary, we can agree with Frenkel (51) who says: »Dermal hypersensitivity is not proof of infection. It may mean persistent infection in certain instances and past infection in others. It is limited in its use to chronic or cured infections of a few hosts as man, monkey and guinea-pig. It is not known whether 'nonspecific' positive reactions ocour.»

CORRELATION AND RELIABILITY OF THE SEROLOGIC METHODS

As will be seen from Table 7, the incidence of toxoplasma in the different countries, according to serologic investigations, varies greatly. This is only to be expected, as toxoplasmosis is believed to be a zoonosis, and a certain connection with infected animal populations and their distribution areas is natural. That toxoplasmosis nevertheless seems ubiquitous can be explained by the marked lack of host-specificity of toxoplasma; in addition, the infection-spreading mechanism, so far unknown, may play a part in this.

Another factor affecting the divergence of results is that toxoplasma antibodies have been measured by such different serologic tests as the neutralization methods with rabbits and with embryonated eggs (NT), the dye test (DT), complement fixation reaction with different antigens (CF), and the so-called toxoplasmin test with different toxoplasmin preparations (SF). Researchers have therefore had every reason to compare all these tests and obtain some idea of their sensitivity. In general, they can probably be placed in the following descending order of sensitivity: DT, ST and CF. (NT, as mentioned, is no longer in routine use.)

Correlation of the Methods

Adams et al. (2) had 89 per cent agreement between NT and ST, and 94.5 between ST and DT. They say: »The agreement between these tests is fairly striking, except for the complement test.»

Frenkel (48) showed that 75 per cent of the ST-positive were positive with NT too, and that 82 per cent of the ST-negative

were negative with NT also. NT often gave results difficult to interpret.

Hogan (75) had assumed in the meantime that NT and DT measured different antibodies. NT antibodies were considered thermolabile.

Winsser & Makstenieks (187) showed that NT antibodies are thermostabile and that a thermolabile supplementary factor must be present in NT for the reaction to function.

Feldman & Sabin (43) found that the agreement between ST- and DT-positive in one and the same material was good. 100 per cent of the ST-positive were also DT-positive. Of the ST-negative 39 per cent were DT-positive. They say: »The toxoplasmic skin test antigen is a soluble substance present in abundance in infected exudate and apparently also proportional to the concentration of the complement-fixing antigen.» They state that no correlation exists between the antibody content in the blood and the strength of the skin test, not even with different toxoplasmin concentrations. For this reason they only use a dilution of toxoplasmin (1: 100) in routine examinations. They point out: »The toxoplasmic skin test is not useful for diagnosis because a strongly positive test bears no relationship to titer of antibody and a negative test is encountered too often in the presence of antibody.»

Fisher (44) examined 100 patients with CF and DT, compared the findings with an ST examination and found that the correlation was sufficient to warrant the use of ST in epidemiologic studies.

Verlinde & Makstenieks (171) found that 60 per cent of 64 toxoplasmosis suspects were positive to both DT and CF. (DT titre between 1/100 and 1/8096, CF between 1/2—1/256; 34 per cent showed DT $\geq$ 1/100 and were CF-negative, and 6 per cent showed DT $<$ 1/100 and were CF-positive.)

Gard (52) reported the following ratio between ST and DT in 310 pregnant women:

DT-positive and	ST-positive	34.8 per cent
DT-positive	ST-negative	7.4 per cent
DT-negative	ST-positive	4.5 per cent
DT-negative	ST-negative	53.3 per cent

Thalhammer (160) had 96.4 per cent agreement between ST and DT. He did not find ST suitable for children under 2 years as they do not react to toxoplasmin. The same applies to the aged as their skin no longer reacts like that of younger people.

Vivell (190) had 95 per cent agreement between DT and ST. He observed a case where a nine-month old child with chronic or subchronic toxoplasmosis still reacted positively with ST when the toxoplasmin dilution was 1: 10,000 (DT = 1: 1600). This seems to suggest that children even become ST-positive if the disease is protracted enough.

The investigation by Humphries et al. (81) showed also that DT-positive children seldom are ST-positive.

Otten (119) investigated 1,284 sera with DT and CF according to Westphal. He obtained 60.2 per cent concurrent results and 28.8 per cent contradictory results; in 10.8 per cent both tests were plus-minus. Of a total of 1,552 sera, 268 showed self-inhibition with CF. He assumed that CF reflected active toxoplasmosis better.

Weigand & Gleim (167) have tabulated their own investigations and those by Westphal & Bauer (176) to show the relationship between DT and CF according to Westphal.

Number of tests	Complete correlation	Poor correlation	Contradictory results	
(115)	87.5	9.0	3.5	Westphal group 1
(170)	82.3	9.5	8.2	„ „ 2
195	84.6	6.7	8.7	
245	84.0	6.5	9.5	
282	81.5	8.5	10.0	
345	76.0	14.0	10.0	

Weigand & Gleim applied the following criteria for positive DT. DT $\leqq$ 1/12 is negative, DT = 1/36 $\pm$, DT $\geqq$ 1/72 are positive. Westphal, on the other hand, has DT = 1/25 $\pm$ and DT $\geqq$ 1/50 positive.

Hein (72), examining patients with tuberculosis, found the following correlation between DT and CF according to Westphal:

CF pos.	64 per cent	DT pos.	56.8 per cent
CF ±	11 per cent	DT ±	9.7 per cent
CF neg.	16 per cent	DT neg.	33.5 per cent

Tilling (cit. by Westphal & Bauer 176) examined 392 persons (mainly children) with ST. He used Westphal's antigen as toxoplasmin. He found 66 per cent agreement with CF according to Westphal. Tilling believed that the ST reaction vas due to humoral antibodies. Out of 13 definite cases of toxoplasma, CF was positive in 10, and 5 out of the 9 examined reacted positively with ST. The mothers of all these children were strongly ST-positive.

Rieger (133), in a material of 143 patients with eye diseases, found 82 per cent agreement between ST and DT.

Keller & Vivell (93) examined 212 persons with DT and ST. The result is shown in the table below.

Infants under 1 year	80	ST	0 per cent	DT	23.8 per cent
1—10 years	49		12 per cent		12.2 per cent
11—20 years	27		44 per cent		48.1 per cent
21—30 years	10		62 per cent		65.0 per cent
31—40 years	16		75 per cent		75.0 per cent

Garin (56) showed that ST according to Frenkel and ST according to Westphal had 82 per cent correlation, and that CF concured with ST in 81 per cent. Garin considered ST to have no diagnostic value.

According to Siim (151), there is no considerable difference between the various CF antigens. Broadly speaking, they give an equal number of positives in one and the same material.

Bleier et al. (17) made a statistical study of ST, DT and CF in materials from Hamburg and Bonn. The material from Hamburg showed good conformity between DT and CF. No conformity was found between ST and CF, or between ST and DT. The Bonn material revealed a weaker, yet definite conformity between CF and DT; the conformity was better among women. The Bonn material showed a weak agreement between ST and CF, and between ST and DT. The differences in the results obtained with the Hamburg and Bonn materials were assumed to be due to the lack of homogeneity of the antigen batches.

Scholta (148), who on Westphal's recommendation used inactivation for 16 hours at +56° C, found the following correlation between CF according to Westphal, and DT.

DT pos.	CF pos.	8
DT neg.	CF neg	21
DT pos.	CF neg.	32
DT neg.	CF pos.	22

Eight sera that were positive in the Gruber-Widal reaction, with titres of 1/400—1/3200, were CF-positive too. He could show that ST gave a positive reaction with typhus patients also. He believed that there may exist antigenic relationships between toxoplasmata and bacteria of the Salmonella group.

Afzelius-Alm & Hahn (4) consider that an antigen according to Westphal reacts non-specifically more often than an antigen made on chorioallantoic membranes.

Present Investigations into the Correlation Between DT and CF

Table 6 gives the titre values for 1,250 sera tested with both CF and DT.

Table 6

The titre distribution of 1250 sera in DT and CF

Dye test reaction

Complement fixation reaction	Neg.1:	4	16	64	256	1024	4096	16,386	65,536
Neg 1:	844	59	60	56	17	6	2		
2	29	10	12	15	14	5	1		
4	15	5	11	18	10	1	1		
8	8	1	3	5	9	2	3	1	
16	5		1	1	5	3			
32			1		1	3		1	
64						1	1		
128				1	1				1

If the material is treated with the rangcorrelation test worked out in 1904 by Spearman (152) for other purposes, the correlation value obtained is $\varrho = 0.727$. Mathematically, the correlation between the two tests, therefore, is good.

Bleier et al. (17) were the first to compare different serologic toxoplasma reactions with Spearman's rangcorrelation test.

The table also reveals that the DT is more sensitive than the CF reaction. The titre values are more concentrated in the upper part of the table; there are 200 DT-positive that are CF-negative, against only 57 CF-positive that are DT-negative. The table shows, further, that a high titre value in the CF test is generally concomitant with a high titre value in the DT test. The opposite does not hold good to the same extent; that it does not may be due to the presence of sera from patients with acute toxoplasmosis. What, then, constitutes high DT and CF titre values? On the basis of the table, the author is inclined to consider CF titre values $> 1/16$ and DT titre values > 256 as high. It must be emphasized, naturally, that this is true only of the present CF and DT tests.

Reliability of the Methods

The CF test with egg antigen is usually recognised as quite specific provided the controls give no cause for objection, i.e. the human serum is not anticomplementary and does not react with egg proteins. The literature contains few reports on a positive CF reaction with diseases other than toxoplasmosis. Ehrmann-Binder (36) showed that sera with toxoplasma antibodies can give non-specific Wassermann reactions. Westphal (174) reports that the antigen he prepared can react non-specifically in *Trypanosoma lewisi* and T. *cruzi* infections. He found a high percentage of positives in ruminants and assumed it to be due to infection by *T. theileri* or *T. melophogium*. Götze (176) showed by infecting calves with toxoplasma that CF according to Westphal is specific with them also. As mentioned previously, a number o authors consider Westphal's antigen unreliable (4, 148). The present author could not use a Westphal antigen manufactured in 1953 as it failed to fix the complement in the presence of immune serum. The present author and Salminen (63) found no correlation

between trypanosomata in the blood of Rattus Norvegicus and positive DT or CF (egg antigen).

It is agreed that CF is less sensitive than DT (106, 140). As a rule, therefore, there are fewer CF-positives than DT-positives. The CF titre values are usually 1—4 tubes lower than the DT. CF antibodies develop more slowly than DT antibodies and disappear faster from the blood (140). For these and other reasons, Feldman (42) claimed that CF was less suited to epidemic investigations. As to toxoplasmosis, Sabin et al. (140) said that CF and DT together gawe a better idea of the course of the disease. Westphal (147) said that CF reflected active toxoplasmosis better.

The present author also holds that even if CF is less sensitive it has a certain value in epidemiologic studies and for comparing the various groups (see also further below). Gard (54) reported having obtained with CF results difficult to interpret.

Guinea-pig studies by Warren & Cutchins (34) have an interest of principle in this connection. They showed that CF antibodies do not develop in guinea-pigs until after the second week. And then only provided the guinea-pigs could be prevented from succumbing to the infection. This was achieved by the use of small infection doses and by the simultaneous administration of suitable doses of sulfadiazine (166). (In these experiments too DT antibodies develop quickly.) Hence it is to be expected that a subacute toxoplasmic infection, if it persists long enough, gives measurable CF antibodies in the blood. By contrast, too small an infection dose often fails to result in measurable CF antibodies. The present author together with Salminen, for example, has shown that in Rattus Norvegicus a certain correlation exists between the amount of the infection dose and the titre of DT antibodies. The maximum dose employed was 500,000 toxoplasmata per rat, and this dose gave no measurable CF antibodies.

If the incidence of human infection is as high as is often claimed it would be quite natural for a number of these infections to be of the type described above, i.e. mild infections. This, together with the fact that CF antibodies develop later and disappear sooner, helps to explain why any given material contains fewer CF-positives.

Warren & Cutchins (34) showed that CF antibodies develop very

quickly on »challenge» in guinea-pigs vaccinated with killed toxoplasmata plus adjuvant and in guinea-pigs with a previous cured toxoplasmic infection. If the same can be assumed to occur in man it would imply that CF antibodies develop more readily on re-infection. This, too, in the present author's opinion, seems to support the fact that CF has its place in epidemiologic studies also. Theoretically at least, it is possible in this way to obtain an idea of how exposed different groups of people are to re-infection.

Warren & Cutchins (34) concluded in their work referred to above that a soluble fraction of toxoplasma was poorer as a vaccine than the whole toxoplasma organism or a major fragment of it. This, however, is true only of CF antibodies. This seems to suggest a greater specificity in CF than in DT antibodies. (This problem will be discussed further below.) Warren & Cutchins showed again the already known fact that toxoplasma can be isolated from animals with a high antibody content and that toxoplasmaemia can be observed in these animals. They assume that antibodies are more protozostatic than protozoicide. That an immunity mechanism other than the humoral also is active is evident from the fact that only living toxoplasmata were able to protect the guinea-pigs in a »challenge» infection of the brain, which may indicate a certain »cell immunity». A »cell immunity» has also been noted by Vischer & Suter (188) on toxoplasmic infection of tissue cultures with macrophages.

Opinions concerning the specificity of the DT reaction differ widely. Kudicke & Pöhlig (99) claimed that experts on toxoplasmosis and toxoplasma problems, such as Sabin & Feldman, van Thiel, Winsser, Bamatter, Westphal, Thalhammer etc., have repeatedly defined their view on this problem and that they do not call the specificity of the DT reaction in doubt.

For instance the fact that ST and DT have such close correlation has been advanced as evidence to show that DT and ST are specific. As a matter of fact, superficially at least, they are two immunologic methods different in principle, and as they do give concurrent results there is some suggestion of a specificity both in ST and DT. The same applies, in some degree, to CF. Frenkel (49), for instance, says: »The presence of complement-fixing antibody and of dermal hypersensitivity may lend further assurance to the

specificity and validity of a dye test titer, although they do not imply current infection.» In the same context he says also: ». . . antibody maesured by test dye provides good indications of infection if high titers are present or if a rise in titer is observed. The interpretation of low titers, 1: 128 and below, is difficult. Many of these can be shown to be due to toxoplasmic infection by isolation of toxoplasma. Others may remain after infection is cured, and some may be 'nonspecific'.»

Keller & Vivell (93) stated that persons with a DT titre of 1/2 showed such a strong reaction with ST that the toxoplasmin could be diluted to 10^{-} without the reaction turning negative. On the other hand, DT-negative persons failed to react to ST with a toxoplasmin solution of 1: 100 even. They assumed this to indicate both DT and ST specificity. They pointed out that investigations revealed a higher number of positives in the environment of toxoplasmic patients and animals with toxoplasmosis than among control groups. Similar environmental studies were made by Feldman (42). He found that one and the same animal species in a certain locality could show 49 per cent positive reactions while elsewhere the same species was completely negative. He also took this to indicate the specificity of the DT reaction.

Among the earliest works that may be considered to suggest non-specific DT reaction may be mentioned the investigation in Germany by Hein (72) of patients with tuberculosis. The tuberculous patients showed a higher number of positive reactions in both DT and CF than the corresponding controls. The same was observed by Wildführ (182) whose material contained a statistically significant majority of DT-positives among tuberculous patients compared with a control material. He was able to show experimentally that tuberculosis in guinea-pigs gave positive DT reactions.

Keller & Vivell (93) observed several DT-positives among children with tuberculous meningitis and believed that toxoplasma possibly can clear a way for the tuberculous process to reach the cerebral membranes. They assumed that tuberculosis may activate an old toxoplasmosis (»Begleittoxoplasmose» by Franke and Horst, 46). In this connection it must be pointed out that Paul (123) isolated toxoplasma from a case of tuberculous meningitis.

Adams (1) stated that he found surprisingly many DT positives among pregnant women and children with rheumatoid arthritis.

Mühlpfordt (113) showed that DT became »non-specifically» positive if guinea-pigs, rats and hamsters were infected with *Sarcocystis tenella.* Avad & Lainson (9, 10) were able to confirm Mühlpfordt's finding. Avad (7) showed later that *Sarcocystis tenella* spores could replace toxoplasmata in DT and that the reaction even then was positive with toxoplasma-immune serum. He recommended these spores for DT as they are less dangerous than toxoplasmata.

Michalzick (111) found that 64 per cent of 50 women with *Trichomonas vaginalis* had DT $\geqq$ 1/15. 22 per cent of them had $\geqq$ 1/50 and 16 $\geqq$ 1/100. CF (Westphal) concurred with these results. Michalzick assumed DT and CF to be non-specific, to be considered rather as group tests. Avad (8) showed by the experimental infection of rabbits and mice with *T. vaginalis* and *Trypanosoma cruzi* that these infections gave non-specific DT reactions. He said: »Therefore the validity of the test in the diagnosis of toxoplasmosis is very dubious», and added: »... the epidemiological surveys based on the dye test will now have to be assessed afresh».

As is evident from the present author's (60) experiments with properdin, it is possible that the properdin system can give positive toxoplasmata in DT.

Further studies are required in order to clarify the final significance of DT and the mechanism involved, which may prove to be of general serologic interest.

PERSISTENCE OF ANTIBODIES IN THE BLOOD

It has been shown repeatedly in most varied experiments that toxoplasmosis is reflected in the serologic reactions. This notwithstanding, toxoplasma has been isolated from animals and men even though simultaneous serologic reactions have been negative (193). Franke & Horst (46) reported a case where DT did not turn positive until after 8 weeks (case 5). They isolated toxoplasma from liquor. Wildführ (185) reported 8 cases with negative DT when animal passage for isolation of the toxoplasma began. In

two cases toxoplasma was isolated from the eye of a child over 1 year old. The balance consisted of younger children from whom toxoplasma was isolated from the liquor. Three of these six children died before long. The cases reported above seem to have been so acute that antibodies had had no time to develop. Frenkel points out, however, that there doubtless are cases of chronic toxoplasmosis with negative serologic reactions.

It may be concluded that toxoplasmic infection in both animales and man is reflected in the serologic reactions. The cases observed in man, admittedly, have been but few, especially as far as adults. of the greatest interest epidemiologically, are concerned. A few laboratory infections have been reported but in these cases the infection route has usually been »abnormal» (157).

The few laboratory infections A) and the parasitologically verified cases of toxoplasmosis B) have a special interest for determining when DT and CF anbodies develop and how long they persist. A clear idea of this is particularly important for epidemiological investigations and at the same time we get a conception of the titre value in DT and CF in human toxoplasmosis. However, it must be emphasized — as the chapter on DT made clear — that the titre values obtained by the different authors are very varying.

Apart from the above groups A) and B), antibody content and »persistence» can also be studied in mothers who have given birth to toxoplasmic babies.

Two cases reported by Gard (52) can be taken as examples of Group A). In one of them, published by Ström (157), the DT titre dropped heavily from the 10th—13th month after the infection and the CF titre from the 9th—10th month. In the other case the DT titre dropped in the 12th—13th month and the CF titre, which had dropped in the 10th month, rose slightly by the 13th month.

Sexton et al. (150) reported a laboratory infection with a fatal outcome. Before the onset of the disease the patient showed negative serologic reactions. On the fourth day of the disease DT was 1/16, on the sixth day 1/64, and on the eighth 1/256. These authors say: »A review of the literature reveals only four authenticated cases of adult toxoplasmosis.» However, several cases have been reported since, but as a rule they have not been followed up for

any prolonged period by serologic check-ups. This applies e.g. to the 14 parasitologically verified cases published by Franke & Horst, with one or two DT values per patient.

Kass et al. (91) reported a case of toxoplasmosis in a woman of 60 who was ill for just over a month. On the 14th day of the disease she was DT-negative, and on the 73rd day the DT titre was 1/32,768.

In his material referred to above, Wildführ (183) noted the following titre values:

8 DT-negatives (in one of them the titre rose in 7 months to 1/12. Ten months before the attempted isolation, another case had been DT-positive with a titre of 1/25 but was negative at the time of the isolation).
4 cases were DT = + 1/4 (One case rose to 1/50 in 10 months).
10 cases showed a titre of 1/12.
3 cases showed a titre of 1/25.
In one case the titre rose from 1/50 to 1/400 in a few weeks.
In another case the titre rose in a month (after treatment) to 1/3,200. The highest titre value was 1/12,000.

There is hardly any doubt that mothers of children with congenital toxoplasmosis are also toxoplasma-infected. These mothers also constitute from the serologic point of view an appropriate investigation group (C). An investigation of this group by Frenkel & Friedlander (51) showed clearly that both DT and CF titres were higher when the child was born (actual infection of the mother too?) and that a follow-up showed declining titre values in practically all the cases. The longest observation period was approximately 6 years. They pointed out that Sabin's (138) results agree with theirs.

Wildführ's (183) material included:

2 mothers with DT titre 1/5 (in one case the titre rose in a year to 1/50).
9 mothers with DT titre 1/25 (in one case the DT rose in a few months to 1/100, in another in a few weeks to 1/50. In 2 cases the titre dropped within a few weeks to 1/12 and 1/5, respectively).
6 mothers with DT tire 1/50.
1 mother with DT titre 1/800.
1 mother with DT titre 1/1,600.
4 mothers DT-negative.

The material available is much too incomplete to give any idea

of the length of time during which DT and CT antibodies persist in the blood of a patient who has had toxoplasmic infection. However, it can be considered as certain that DT antibodies develop sooner and disappear later on infection than CF antibodies (140). Titre values exceeding 1/265—1/1,024 can hardly be observed in the blood for any considerable length of time, perhaps for a maximum of 2—3 years. On the other hand, titre values on the borderline of the measurable or somewhat higher are more frequently noted after parasitologically verified cases of toxoplasmosis.

THEORETICAL SPECULATIONS REGARDING THE ANTIBODIES IN TOXOPLASMOSIS AND THE MECHANISM IN THE DYE TEST

DT and CF antibodies have generally been assumed to be two different antibodies. Wildführ (183) however, emphasized that there need not necessarily be two different antibodies; it may be a question of two different antibody functions of one and the same antibody. The experimental conditions and the conditions of the in vitro tests produce the differences. It is also natural to assume two »multicatalytic centres» (Sevag 149) in one antigen molecule. The one inducing DT antibodies can be assumed to lie more superficially while the one inducing CF antibodies is situated deeper in the antigen molecule and hence forms antibodies more poorly. A certain activity of the toxoplasma (acute infection?) and of the defence mechanism is required before CF antibodies are formed. For this reason they occur later and disappear sooner. The relationship between the antibodies and the placenta, discussed on pp. (71—74) can be taken to suggest such a state of affairs. From a wider point of view, it seems beyond doubt that the toxoplasma in its entirety induces different antibodies. It is possible that the CF and DT antibodies are the actual two different antibodies. The experiments by Warren & Cutchins (34) seem to suggest this. They showed that a toxoplasma changed as little as possible was the best antibody inductor for the development of CF antibodies. The soluble antigen, also complement-fixing, was exceedingly poor in inducing CF anti-

bodies. On the other hand, the soluble fraction, and likewise any component of the toxoplasma, was strongly DT antibody-inducing. This can be taken to suggest that two different antibodies are active in DT and in CF. However, Weinman's (169) toxotoxin can hardly be involved. There can just as well be similar decomposition products from toxoplasma or other protozoa. The fact that Visher & Suter (188), on staining tissue culture, some time after its infection with toxoplasma were unable to find toxoplasmata with stained cytoplasm perhaps suggests that toxoplasmata need not necessarily be dead to yield the antigen concerned. This antigen gives rise to antibodies that may be called »cytoplasm-modifying antibodies» (CMA). It is difficult to believe, however, that completely »whole» toxoplasmata are CMA-inducing as the antigen inside the toxoplasma cannot come into contact with the antibody-producing centres. Whole active toxoplasmata (infection!) and their fragments can be taken to serve an adjuvant for CMA induction. Infection is apparently the best inductor of antibodies. What »determinants of antigenic specificity» (Sevag) are required to induce the development of CF antibodies is unclear. CF antibody formation is usually slower and can probably seldom be invoked in infectious diseases by anything but infection. Warren & Cutchins (34) succeeded, as pointed out, in producting CF antibodies with an adjuvant and toxoplasma. They assumed that it was the first time that anybody had succeeded in producing them without infection.

Toxoplasma is evidently a microorganism relatively apathogenic to many animals and to man, with markedly commensal habits of life. If toxoplasmata can only propagate inside living cells it is possible that the CF antigen cannot escape from the cell to induce the formation of CF antibodies. This occurs only when toxoplasmata are present in profusion in the surrounding tissue fluid, i.e. in acute infection (dualism of toxoplasma?).

The possibility cannot be excluded that CMA-inducing antigen can give rise to CF antibodies in particularly favourable conditions. In the experiments by Warrens & Cutchins, two out of five guinea-pigs developed CF antibodies, with an adjuvant and soluble antigen, at a low titre. This suggests two types of antibodies of which the one against the CMA antigen is less specific.

It seems obvious that *Sarcocystis tenella, Trichomonas vaginalis,* trypanosomes and possibly also tuberculosis can give rise to CMA which affect toxoplasmata and sarcocystis spores in DT. Hence the CMA, for toxoplasma, are defintely non-specific. Superfically seen, it is easier to understand the common reaction to the protozoa in question — perhaps a kind of group test? But how tuberculosis develops CMA is more difficult to understand. It must be emphasized in this connection that it of course is possible that animals or men with a tuberculous infection may have had toxoplasmosis earlier which was serologically inactive and was now activated. The final consequence is, however, that the tubercle bacillus together with the host cell forms an antigen-producing unit which induces CMA formation (a sort of »auto-antibodies»).

If, as assumed above, there are two types of CF antibodies it is possible that a more specific antigen could be obtained by pre-treating the toxoplasmata to remove the soluble antigen. This can be achieved by using the centrifugate from DT-positive tests by treating the toxoplasmata with distilled water according to Bringmann & Holz (18) or by treating them with properdin according to the present author (60). The method of Behrens & Geissler (14) may perhaps be usable. The extensive survey of the different antigens (pp. 20—25 above) shows clearly enough that the antigens used contain a considerable amount of precisely this soluble antigen, and to a varying degree possibly »stroma antigen». The most suitable antigen from this point of view is perhaps that of Steen & Kåss, and possibly chorioallantoic antigen. Sabin's peritoneal exudate antigen is the least suitable. It is not impossible that Westphal's antigen is a pure »CMA antigen». This might explain why Westphal himself obtained a high correlation between CF and DT, and why Hein & Scholta had non-specific reactions. Westphal himself also had non-specific reactions in trypanosoma infections. This pure CMA antigen can be assumed to react with complement-fixing CMA which, though difficult to induce, are present in prolonged and serious infections.

Sabin & Feldman state that DT antibodies bind themselves to the toxoplasmata even in the absence of AF. If the antibodies in this case are taken to be CMA the binding cannot be particularly strong as CMA cannot pass through the surface of the toxoplasma. The

antibodies directed towerds the surface layer can naturally be assumed to attach themselves well (CF antibodies?). Toxoplasmata with such an antibody shell are virulent for mice, which seems to indicate that the fixation is not particularly strong. It has long been known that specific immune sera against protozoa have a paralysing effect on their motoric-trophic centres (134, 35). This process also occurs in the absence of complement to fairly high dilution of the immune sera (1/800—1/3,200) (134). If compiement is added the whole process is very rapid but the titre value remains unchanged. Harrison & Fowler (67) were able to show that also a heavy dilution of immune sera against the ciliates of tetrahymena had an inhibitory effect on its breeding. A similar effect is ascribed to sera immune against toxoplasma also. As has already been pointed out, Warren & Cutchins believe that toxoplasma immune serum is more protozostatic than protozocide. If CMA is allowed to act on toxoplasma at close quarters the toxoplasma is irreversibly injured. It is obvious that this effect is produced by AF. AF, as pointed out above, is equal to properdin + C'_2 + C'_3 + C'_4. It seems as if properdin attaches itself to polysaccharides in e.g. the red cells of patients with paroxysmal nocturnal haemoglobinuria in the same way as it attaches itself to the insoluble polysaccharide remnant of yeast (zymosan). In patients with the above disease the properdin system produces haemolysis (73). It is to be expected that the properdin effect on toxoplasmata is of the same type and has the same results in relative toxoplasmolysis. An adequate properdin content alone can perhaps lyse the toxoplasma when the C'-components are present. That ordinary serum protozoicide has long been established. A serum dilution 1/40 is enough to leave a considerable number of the protozoa unaffected. The titre corresponds to that observed by Jettmar (89) for his »toxoplasma hostile factor». If ordinary normal serum is inactivated it loses its protozoicide effect. Only after Pillemer et al. (131) discovered properdin did this phenomenon become more readily understandable. The mechanism proper, however, is still unclear. Toxoplasmolysis may be assumed to be similar to haemolysis which, as is known, can occur without the stroma of the red blood cells changing. The same result can be achieved by treating the blood cells with distilled water or with diluted solut-

ions of invert soap. Toxoplasmata also can be changed with distilled water (18) and with invert soaps (61, 51). The present author even managed to obtain a certain, although insignificant AF serum effect in DT with a diluted invert soap solution (61).

It is possible to standardize DT e.g. by using pure properdin. However, it seems pointless to employ DT on a large scale before we know the biological effect of CMA.

The great concurrence between the DT- and ST-positivity of persons whose skin is highly sensitive to toxoplasmin has been referred to as evidence of the specificity of DT and ST (93). That this could also serve to prove the specificity of skin reactions in which a high toxoplasmin concentration is used does not follow quite so simply. On the contrary, the problem must be attacked with new energy. This also seems to apply to the specificity of tuberculin reactions with concentrated tuberculin. In this connection an American investigation made into tuberculin sensitivity to varying doses of tuberculin is of importance. It turned out that the geographical districts in which the sensitivity to diluted tuberculin doses was high also showed high tuberculous morbidity (122). On the other hand, there was no correlation between sensitivity to a high tuberculin concentration and morbidity. A similar state of affairs was reported from the hill districts of India regarding the dependence of tuberculin sensitivity on the altitude of residence (13).

Before the toxoplasmin reaction can be shown to be specific these must be definite evidence that no Shwartzman reaction, for instance, is involved in the higher concentrations.

III SEROLOGIC SERIAL STUDIES OF TOXOPLASMOSIS

and some epidemiologic aspects

REVIEW OF THE LITERATURE

For easy reference the literature on earlier serial investigations of toxoplasmosis is reviewed in tabular form. As far as possible the table 7 includes all investigations into the serologic epidemiology of toxoplasmosis and, in addition, individual special serial investigations.

In the last column of the table 7 the following symbols are employed: NM = normal material, i.e. blood donors and otherwise healthy persons, HM = hospital material, i.e. patients hospitalized for various complaints, though not suspected of toxoplasmosis, SM = selected material, i.e. patients with certain types of diseases.

The serologic methods, as throughout the present work, are indicated as follows: NF = neutralization test, DT = dye test, and CF = complement fixation test.

For instance, DT + $\geq$ 1/50 in the last column indicates that the author in questions makes a dilution of 1/50 the limit for a positive dye test.

N.B. SECTION III (pages 61—99) OF THE PRESENT WORK DEALS WITH DT, CF AND ST AS IF THEY WERE *SPECIFIC.*

Table 7

Serological investigations of toxoplasmosis

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Callahan 1945 (21)	St. Louis	17—28	100	2.7	NT		NM
Heidelman 1945 (71)	Baltimore	adult	58	10	NT		NM
Cathie et Dudgeon 1949 (24)	England		200	1.5	CF	both.	HM. 200 sera sent for Wasserman-reaction CF≧ 1/10.
Kabler et Cooney 1947 (90)	Minnesota	differ. ages	761	42.3 (1944) 19.8 (1945) 18.4 (1946)	NT		SM. Highest % of + in chorioretinitis group.
Adams et al. 1949 (2)	Minnesota	differ. ages	188	5—10	NT ST DT CF		SM
Feldman et Sabin 1949 (43)	Cincinnati	0—4	20	0	ST	Men 0/12 Women 0/8	NM. Mean value 31 % positive.
		5—9	20	5		0/12 1/8	
		10—19	20	14		0/8 3/12	
		20—29	20	20		2/14 2/6	
		30—39	20	50		7/13 3/7	
		40—49	20	65		9/15 4/5	
		50—81	20	65		10/17 3/3	
Frenkel 1949 (48)	San Francisco	20—35	50	10	ST		HM
		30—83	50	28	a.		HM (chorioretinitis).
		mean 23	28	71	NT		
		mean 35	40	33			HM (anter.uveitis).
Holmdahl 1948—49 (76)	Gothenburg		240	55.4 (5.0)	NT	Only women	NM (deliveries).
			120	55.8 (5.0)			SM (women with perinatally dead children).
			134	53.0 (3.0)			SM (women with premature deliveries).
			102	56.9 (2.0)			SM (women with abortions).
			98	54.1 (2.0)			NM (women with no pregnancy).

In parentheses, per cent of sera with titre ≧ 1/100.

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Roth et Fritz 1950 (136)	Switzerland		54	22	NT		NM
Verlinde et Makstenieks 1950 (171)	Holland	Children and adults	600	10—11	DT CF	both	Of 64 in a SM 60 % had titres ≧1/100 and were CF-positive. Highest DT titre = 1/8192.
Westphal 1950 (179)	Hamburg		50	0	DT		NM
			103	39			HM
			100	3			NM. (women post partum).
			134	24.6			SM. (mothers of toxoplasma children, people with eye and with mental diseases).
Fisher 1951 (44)	England	0—4	186	1	ST	both	HM and NM. Positives equal in both the sexes.
		5—9	322	4.3			
		10—14	216	7			
		>15	177	17.5			
Gard 1951 (52)	Stockholm	adult	371	45.6	ST	men	NM. The highest per cent of positive tests was in the age group 30-50 (50 %).
		adult	513	46.4	ST	women	
		0—9		0			
		11—15	128	16.4		both	
		8—10	156	14.1			
Kostman 1951 cit.by (52)	Boden Sweden	20—50	173	9.8	ST		NM. Of the positive 8 were from Boden and 9 from south Sweden.
Krieg 1951 (98)	West-Germany and Berlin	1—10	5	40 (40)	DT	both	SM. Only people with inflammatory eye diseases. Posit. roughly equal in both the sexes. The highest DT titre = 1/200 DT+≧ 1/2. In parentheses, per cent of sera with titre ≧ 1/50.
		11—20	41	43.9 (12.1)			
		21—30	78	48.7 (19.2)			
		31—40	52	48.1 (11.5)			
		41—50	52	67.3 (13.5)			
		51—60	39	51.3 (10.3)			
		61—70	15	86.7 (26.7)			
Zeipel et Lindner 1951 (194)	Denmark	mean 31.5	300	36.7 (1.3)	DT	both	NM. Blood donors (90 % males).
			139	36.6 (0.7)	DT	both	Sera sent for Widal reaction.
			164	31.7 (6.1)	DT	both	Sera for »encephalitis reaction».
			280	36.1 (3.6)	DT		Sera for cold-agglutination.

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Authors above		30.5	270	48.5 (3.8)	DT	women	Gravidae.
		27.5	93	54.8 (2.2)	DT	women	Abortions.
			25	64.0 (12.0)	DT	both	Morbus Schauman
			328	38.4 (6.6)	DT	both	Sera sent for DT.
			324	37.9 (6.7)	DT	both	For Paul-Bunnell's reaction.
		total	1923				DT $+\geqq$ 1/10. In parentheses, per cent of sera with titre $\geqq$ 1/250.
Feldman **1952 (39)**	Pitts-burg	0—4		5	ST	both	NM. DT $+\geqq$ 1/16
		5—9		10			
		10—29		25—30			
		30—39		45			
		$\geqq$40		65—70			
Fois et Vullo **1952 (45)**	Sassari, Italy		390	6.7	ST	both	HM. Patients from mental hospital. Posit. equal in every age-group.
Harboe **1952 (66)**	Norway Oslo.	18—77	1599	7	DT	men	NM. Oslo blood donors.
				11	DT	women	He counted 80 % unstained instead of 50 % in DT.
			223	9.8	DT		Sera from healthy persons not living in Oslo. DT+$\geqq$ 1/40.
Hein **1952 (72)**	Hamburg Heidel-berg	10—19	5	60	DT		HM. Patients with tuberculosis.
		20—29	13	46.1	and		
		30—39	13	83.3	CF	men	DT+$\geqq$ 1/50
		40—49	19	47.2			
		50—59	15	40.6			
		$\geqq$60	6	66.6			
		10—19	2	100		women	
		20—29	11	81.8			
		30—39	13	84.6			
		40—49	3	100.0			
		50—59	1	0			
		$\geqq$60	1	100			
Humphries et al. **1952 (81)**	Louisi-ana	0—4	40	15	DT		HM. Children not suspected of toxoplasmosis.
		5—9	45	33.5			
		10—15	17	35			
		0—4	13	7.6	ST		
		5—9	22	22.7			
		10—15	6	83.3			

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Iversen 1952 (83)	Hamburg	50—59		19	DT	men	DT+≧ 1/25.
		60—69	144	20	CF		NM
		70—81		33			
		50—59		40		wo-	
		60—69	66	30		men	
		70—81		80			
Keller et Vivell 1952 (93)	Freiburg / Br.	Newborn	50	74 (54)	DT	both	NM
		1—5 mo.	66	40 (2)			DT+≧ 1/2.
		6—12 mo.	53	4 (4)			In parentheses, per cent of DT-positive in dilution ≧ 1/25.
		1—10	82	28 (17)			
		11—20	127	45 (20)			
		21—30	327	58 (23)			
		31—40	170	62 (22)			
		41—50	74	70 (19)			
		≧50	75	66 (21)			
		Total	1024	53∓ 1,6			
Kunert et Jüptner 1952 (100)	Berlin		1000	1.8	DT		NM. Blood donors.
			137	1.3			SM. People in veterinary work, gravidae with complications, imbecillae. DT+≧ 1/64.
Koch-Institut 1952 (95)	Berlin		1000	2.7	DT		NM. DT+≧ 1/64
			330	5.7	DT		SM. People suspected of toxoplasmosis.
Bader (These by Kühn) 1952 (11)	Heidelberg		1000	11	DT		NM
Piekarski et Peters 1952 (128)	Bonn			7.5	DT		NM. Urban dwellers
				20	DT		Rural dwellers.
		6		0			NM. Urban children
		6—16		5.4	DT		—»—
		6—16		10.5	DT		Rural children.
Rodrigues et Gomez 1952 (136)	Colombia	Adult	30	3.3	DT		NM: Only one positive case, titre = 1/2.
Schmid 1952 (147)	Freiburg		238	53	ST	both	NM.
Westphal et Bauer 1952 (176)	North Germany			18	CF	both	

Author	Place	Age	No. of tested	% positive	Method	Sex	Type of material and notes
Wiesman et Brunner 1952 (180)	Switzerland		225	12	CF		HM.
Buhn 1952 (20)	Freiburg	About the same mean age in each group	118	62.7 (1.9)	DT	women	Women with normal children.
			70	61.4 (3.3)		women	Women with miscarriages and stillborn.
			84	66.7 (2.4)		women	Women with abortions.
			54	63.0 (1.8)		women	Gravidae.
			132	56.1 (—)		women	Healthy women.
			70	55.7 (—)		men	Healthy men. DT+ ≧ 1/2. In parentheses, per cent sera with titre ≧ 1/25.
Biagi 1953 (15)	Mexico	6—8	29	13.8	ST		NM.
		9—10	21	38.1			
		11—19	14	50.0			
		20—29	15	66.6			
		30—39	26	96			
		40—49	8	62.5			
		50—76	14	64.2			
Garin 1953 (56)	Lyon	Adult	105	8.5	CF	both	NM. Blood donors.
			150	10.6	ST	women	Post partum.
		22	31	6.4	ST	women	
		14	119	11.8	ST	women	
			56	8.9	CF	both	Neurologic cases.
			56	32.0	ST	both	Neurologic cases.
			51	23.5		children	Chorioretinitis.
			14	18.5		— » —	Encephalopathie.
			63	14.0	ST	men	Mentally sick.
			63	6.3	CF	men	Mentally sick.
		5—18	163	20.0	CF	both	Children with psychic disturbances
			85	24.2	CF	both	
			78	15.3	CF		Debila.
Hagberg 1953 (65)	Stockholm	7—8	114	8.8	ST	both	NM. No sex difference has been noted.
		9—10	174	14.9			
		11—12	99	16.2			
		13—14	43	18.6			
Hedqvist 1953 (70	Eskilstuna	0—4	201	0	ST	both	NM. Egg-toxoplasmin. Most of the people in age groups over 15 years are women.
		5—9	104	1			
		10—14	74	0			
		15—19	73	22			
		20—29	122	34			
		30—50	74	47			
		(20—50)	196	40			

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Horstmann et Kraft 1953 (77)	Tahiti	All age groups over 5 yr.		75—90			NM.
Otto 1953 (121)	Leipzig		125	6			NM. DT+≧ 1/50.
Kozar 1953 (97)	Gdansk	18—60	690	25	ST	both	NM. More young people 20-25 yr.
		18—30	245	40.4	ST	women	NM. But from »toxoplasmic environment«.
		Different ages	446	17.1		both	NM. Mostly manual workers.
Pigeaud 1953 (130)	Lyon	22	31	6.4	ST	both	NM.
		22	119	11.8		both	NM.
		93	93	12.9			NM. Urban dwellers
			54	7.4			NM. Rural dwellers
			8	25.0			SM. Mothers with stillborn.
			7	14			SM. Praematurе babies.
			12	16.6			SM. Obstetrical cases.
			26	11.1			SM. Mothers with babies over 3 kg.
			150	10.6			NM. Obstetrical clinic.
Thalhammer 1953 (162)	Vienna	0—5	61	0	ST	both	NM.
		6—10	90	10.6			
		11—20	116	28.4			
		21—30	311	62.1			
		31—40	158	70.3			
		41—50	85	78.3			
		≧50	185	73.6			
		Total	1006				
		0—5	207	0.9	DT	both	NM. DT+≧ 1/2.
		6—10	66	9.0			
		11—20	76	36.8			
		21—30	129	51.1			
		31—40	99	62.6			
		41—50	89	64.0			
		≧50	115	76.4			
		Total	781				

Author	Place	Age	No. of tested	% positive	Method	Sex.	Type of material and notes
Kudicke et Pöhlig 1954 (99)	Saxony	0—12 mo.	19 (120)	— (55.8)	DT	men	NM. In the first column are the number of sera in a separate investigation with DT when sera were diluted 1/12, 1/36, 1/72 ... DT+≧ 1/12. In parentheses, the number of sera in a different investigation with DT dilution 1/4, 1/16, 1/64 ... DT+≧ 1/4. The figures in the per cent column are the corresponding per cents.
		1—10 yr.	103 (197)	35.9 (39.1)			
		11—20	47 (98)	48.9 (65.3)			
		21—30	84 (191)	57.1 (59.2)			
		31—40	84 (83)	61.9 (77.1)			
		41—50	80 (69)	77.5 (78.3)			
		≧50	91 (59)	68.1 (98.2)			
		Total	534 (820)	58.1 (59.8) ±6.4 (±5.1)			
		0—12 mo.	19 (120)	— (55.8)			
		1—10 yr	56 (123)	53.6 (41.5)			
		11—20	112 (159)	69.6 (74.8)			
		21—30	189 (106)	71.4 (83.5)			
		31—40	139 (323)	78.4 (87.9)			
		41—50	91 (174)	74.7 (88.5)			
		≧50	94 (69)	79.8 (89.9)			
		Total	700 (1374)	71.6 (78.3) ±5.1 (±3.3)			
Beverley et al. 1954 (14)	Sheffield		DT≧1/4	DT≧1/16	DT	both	NM. There was no sex difference. The χ^2 test showed no significant differences at a titre 1/4 or more between the age groups ≧ 20.
		0—9	108	4.6 1.9			
		10—19	55	45 7.3			
		20—29	101	43 5.9			
		30—39	105	41 1.9			
		40—49	108	34 3.8			
		≧50	104	43 2.9			
Wildführ 1954 (183)	Leipzig	1—10 yr.	75	9.3 6.7	DT	both	NM.
		11—20	143	17.5 2.1			
		21—30	152	28.9 1.3			
		31—40	257	42.4 3.1			
		41—50	380	32.9 1.3			
		51—60	170	38.8 0.6			
		≧60	28	32.1 0.0			
		Total	1205				

PRESENT INVESTIGATIONS

Serologic Epidemiology of Toxoplasmosis in Finland

General Data on Finland (158): Finland is situated between the 60th and 70th parallels, in the north-east of Europe. Excluding lakes and other water, the surface area of the country is 305.396 sq.km. Population in 1953 totalled 4.165.298, of which 1.384.413 were urban dwellers and 2.780.885 lived in the rural districts. The capital of Finland, Helsinki, has 394.736 inhabitants. The country has another two cities with a population of over 100.000. All the other towns have less than 50.000 inhabitants.

In the southern parts of the country the density is 30 inhabitants per sq.km.; if the capital is included this figure rises to 72 inhabitants per sq.km. In the central parts of the country the density is 15, further north 7 and in the northernmost parts 2 inhabitants per sq.km.

Forestry and agriculture are the most important industries in the country.

The Material Studied: In the course of four years the present author has made DT and CF investigations of sera submitted to the Helsinki University Institute for Serology and Bacteriology for other serologic reactions, such as Wassermann, Widal, Waaler-Rose, Paul Bunnell, etc. Sera from blood donors and other healthy subjects have also been studied. As the possibility of making both DT and CF was limited, only some of the sera, as fresh as possible, of the above groups have been examined. When not studied on the day of arrival the sera were kept at —18—20° C. As stated before, DT antibodies keep very long at this temperature (101). But the number of non-specific reactions increases when serum is stored for a long time. The specific CF antibody, however, stands storage.

To ensure that the material studied is as representative as possible of the average population in Finland and to provide the best idea of the serologic epidemiology of toxoplasmosis, the sera from patients with the following diseases were excluded from the groups:

1. Hydrocephalus or microcephalus.
2. Chorioretinitis
3. Intracerebral calcareous foci.
4. Psychomotoric retardation.
5. Mothers of children with the above diagnoses.
6. All types of tuberculosis.

As can be realised from a glance at the above list, it is mainly in the younger age groups that selection has been made. This because congenital toxoplasmosis shows a fairly well defined disease picture to which e.g. diagnoses 1—4 above belong. The existence of these cardinal symptoms of congenital toxoplasmosis, described at an early date, has been confirmed by later, extensive investigations. Feldman (40) found, for instance, that 99 per cent of 103 cases of congenital toxoplasmosis had chorioretinitis, 63 per cent had cerebral calcareous foci, 56 per cent showed psychomotoric retardation, and one-third had hydrocephalus or microcephalus. Eichenwald (37) reported that toxoplasmosis often manifests itself in the form of psychomotoric retardation. Wildführ (183) had 21 patients with hydrocephalus among 39 cases from whose liquor toxoplasma had been isolated, 6 had cramps, debility was a frequent phenomenon, and in 15 cases the patients showed eye symptoms. Thalhammer (162) said of mental retardation: »I am of the opinion that a non negligible percentage of our connatal mental defects and epileptics have to be looked upon as oligosymptomatic connatal toxoplasmosis.»

On the other hand, the material includes a number of children (and their mothers) with congenital malformations. As Bamatter (12), Thalhammer (161), and others have pointed out, the malformations proper, if caused by microorganisms, are probably a result of infections in the first trimenon. This applies particularly e.g. to rubeola and parotitis vira. A toxoplasmic infection in the first trimenon is believed to result in abortion (12, 161, 179). Westphal & Schultz (179) are doubtless over-hasty in making assumptions from too limited a material as to the significance of toxoplasmosis as the cause of abortion. Buhn (20) also found that these authors overshot the mark. Vivell & Buhn (191) were more credible on this point in saying that 1 pro mille of all babies born are toxoplasma-infected. For this reason, mothers with abortions or still-births have not been excluded from the present material.

The present author is aware that the material perhaps gives more DT- and CF-positives than a pure blood donor material would have given. However, blood donors are included in the material and they are also treated separately to obtain control groups.

The notes columns of the tables refer to the composition of the material: NM = hospital patients, with the exceptions mentioned above, plus blood donors. BD = blood donors, i.e. healthy persons SM = selected material, i.e. certain groups of diseases, and mothers of children from these groups.

Table 8 shows the number of DT- and CF-positives in dilutions DT $\geq$ 1/4 and CF $\geq$ 1/2, and the distribution of titres in the relevant tests.

As transplacentally carried antibodies are of a certain importance, not only from the immunologic but also from the diagnostic point of view, the group including blood from the newborn has been dealt with separately. The group is in the first place compared with the under 1 year old age group and with the 1—9 year old group. Sabin & Feldman (142) showed that both types of toxoplasma antibodies are carried through the placenta from mother to child. They found that the titre level in children remained unchanged for six weeks. By the end of the fourth month there was a 32-fold reduction in titre level. Small amounts of DT antibodies could be demonstrated in children at the age of 9 months still.

Buhn (20) is probably the only one who has carried out extensive investigations into the relationship of toxoplasma antibodies in the newborn and in infants. DT was positive in 74.5 per cent of 59 cases in puerperium, and the newborn showed the same percentage. 7 per cent of the mothers and 8.5 per cent of the babies showed titres over 1/100. Even with lower DT dilutions it was often noted that the baby had a higher DT titre than the mother. If the mother was positive the baby was always DT-positive. DT-negative mothers always had DT-negative babies. A control examination of 18 DT-positive babies and their mothers after 4—6 months showed that the titre value of the mothers had remained approximately the same while half the number of the babies had turned DT-negative. The majority of those remaining positive had very low DT titres. A study of 56 mothers and their children whose ages ranged from 4 weeks to 1 year showed a similar state of affairs. Buhn concluded that serologic diagnosis of toxoplasmosis in the newborn was impossible but that a high DT titre after the 4th month of life suggested active toxoplasmosis. The value of the

Table 8

Distribution of the »normal material» by age groups and tests

(see pp. 69—71).

		No. of tested	No. of negative	Titres in dye test (DT) and complement fixation test (CF)								A	B	C
Values of K.				2	4	6	8	10	12	14	16			
Titres in DT.			neg.	4	16	64	256	1024	4096	16389	65536			
Titres in CF.			neg.	2	4	8	16	32	64	128	256			
Values of K.				1	2	3	4	5	6	7	8			
Men	DT	11	6		1	4							46±15	5,60±0,10
umbil. cord blood	CF	15	12	1	1		1						20±10	2,33±0,88
Women	DT	10	6		1	2		1					40±15	6,50±1,26
	CF	13	11	2									15±10	1,00
Men	DT	38	36		1	1							5±4	5,00±1,00
< 1 year	CF	36	36										0	
Women	DT	28	25	1	1			1					11±6	5,33±2,40
	CF	29	27	1						1			7±5	4,00±3,00
Men	DT	89	85	1	1	1			1				4±2	6,00±2,16
1—9 year	CF	103	90	5	4	3	1						13±3	2,00±0,28
Women	DT	60	54	1	3	1	1						10±4	4,67±0,84
	CF	68	55	6	5	1						1	18±5	1,58±0,19
Men	DT	24	20		2	2							17±8	5,00±0,58
10—19 year	CF	36	31	3	2								14±6	1,40±0,24
Women	DT	34	25	2	3	3				1			26±8	5,44±1,20
	CF	50	40	4	2		1					3	14±5	1,71±0,42

	DT	54	25	2	3	3				1			26±8	5,44±1,20
	CF	50	40	4	2		1					3	14±5	1,71±0,42
Men	DT	141	117	4	3	10	4	2	1				17±3	6,00±0,54
20—29 year	CF	202	177	13	7	3			1			1	12±2	1,75±0,24
Women	DT	303	232	12	18	17	16	5	2	1	1		23±2	5,41±0,44
	CF	344	271	21	17	4	4	1	1	1		24	14±2	2,06±0,20
Men	DT	27	18	3	4	2							33±9	3,78±0,52
30—39 year	CF	49	42	3	3							1	12±5	1,50±0,22
Women	DT	198	125	18	21	20	10	4					37±3	4,93±0,27
	CF	206	162	12	13	3	4			1		11	16±3	2,12±0,23
Men	DT	14	9	3	2								36±13	2,80±0,49
40—49 year	CF	36	31	2	2							1	11±5	1,50±0,29
Women	DT	65	39	3	7	4	8	2	2				40±6	6,38±0.57
	CF	82	63	9	6	2	8		1				23±5	1,95±0,30
Men	DT	23	13	1	3	5	1						43±10	5,20±0,53
50—59 year	CF	44	39	3	1							1	9±4	1,25±0,25
Women	DT	52	28	8	7	6	1	1	1				46±7	4,00±0,72
	CF	62	49	7	2	3	1						21±5	1,85±0,30
Men	DT	23	13	8	1	1							43±10	2,60±0,43
≥ 60 year	CF	47	42	5									11±5	1,00
Women	DT	35	24	3	3	4		1					31±8	4,73±0,73
	CF	43	33	5		1						4	14±5	1,33±0,33

Column A shows the number of sera that reacted anticomplementarily or against egg protein in the CF. — Column B shows the per cent ± S.E. of positive in DT and CF. — Column C shows the mean value of the titres ± S.E. in the logarithmic scale 2^{-k}. DT titres have k values 2, 4, 6, 8 . . . and CF titres have k values 1, 2, 3, 4 . . .). — The orginal titres in DT and CF are expressed as reciprocals.

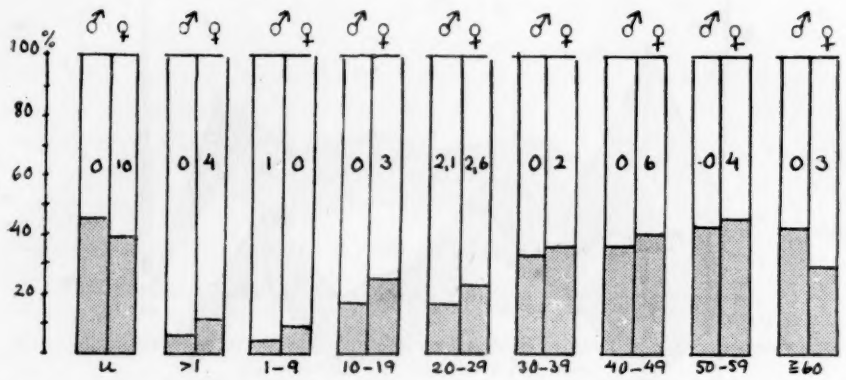
serologic diagnosis declines again after the age of 2 1/2—3 years.

In the present material, the mothers of the healthy babies from whom umbilical cord blood was taken were unfortunately not examined. But the author examined toxoplasmic children of various ages and their mothers. The material is limited, but the result concurs with Buhn's finding that the titre value in these children remains high. The number of positives in the small material in the group newborn (Table 8) is percentually high and the mean titre value in the logarithmic scale (MTL) is also high (Figs. 3, 4, 5, 6). However, the group includes no one with a »high» titre (see p.49). A superficial comparison of the newborn group with women of age group 20—30 shows that the former has a higher percentage of positives than the latter, and this age group includes the majority of the mothers. This can also be interpreted as above, viz. that the newborn have often been found to show higher titre values than the mothers without the babies being infected with toxoplasmosis. It may be due to a placental capacity for actively taking up and conveying to the foetus serum components of importance to it. Longsworth et al. (105) have shown that the gamma globulin concentration in the foetal blood is higher than in the maternal blood. This concurs well with the above facts on toxoplasma antibodies. However, it should be borne in mind that the placenta reacts differently to the different antibodies. Adamson et al. (3) showed that the concentration of H and O antibodies against Escherichia coli is higher in maternal than in child's blood. The month of pregnancy or, in other words, the developmental stage of the placenta doubtless affects the above functions. By contrast, the size of the antibodies seems to play no part (68). As was shown by Sabin & Feldman (142), CF antibodies pass through the placenta as readily as DT antibodies. Hence the method provides no means of showing that DT and CF are two separate antibodies. On the contrary, it may be taken to suggest that they are not, that one antigen has more than one »antigenic group» and that prolonged and sufficiently strong immunization results in the formation of other antibodies, in this case CF antibodies.

A closer study of the newborn group and the women of the 20—29 age group shows, however, no statistical percentual difference between the two. The same applies to MTL, as is clearly seen from

Fig. 1

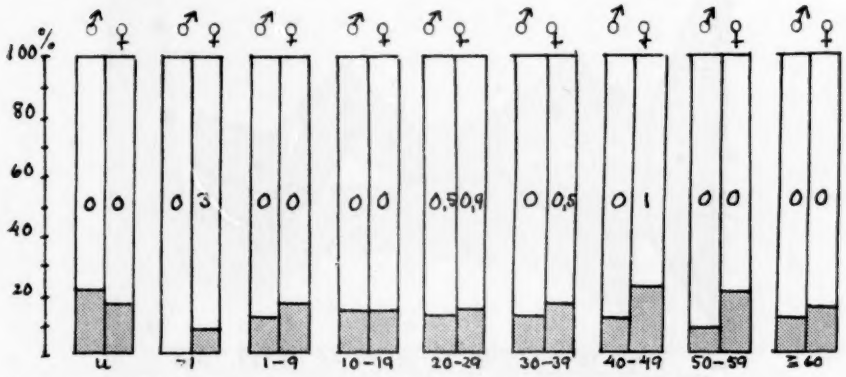
Per cent of DT-positives in the different age groups.



The figures inside the columns give the per cent of sera with a DT titre over 1/256. U = Umbilical cord blood.

Fig. 2

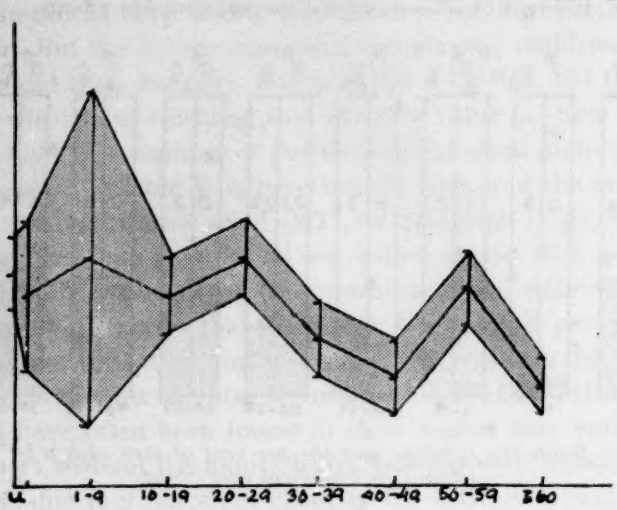
Per cent of CF-positives in the different age groups.



The figures inside the columns give the per cent of sera with a CF titre over 1/16. U = Umbilical cord blood.

Fig. 3

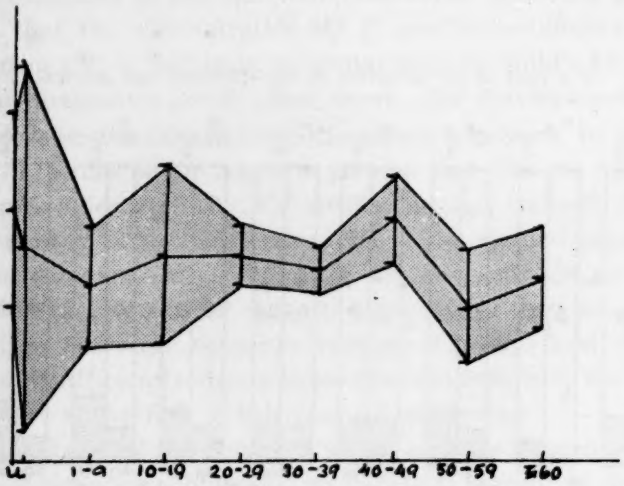
Scale 1/2



Graphic representation of the mean titre values (in the logarithmic scale) of the positive DT reactions for men.

Fig. 4

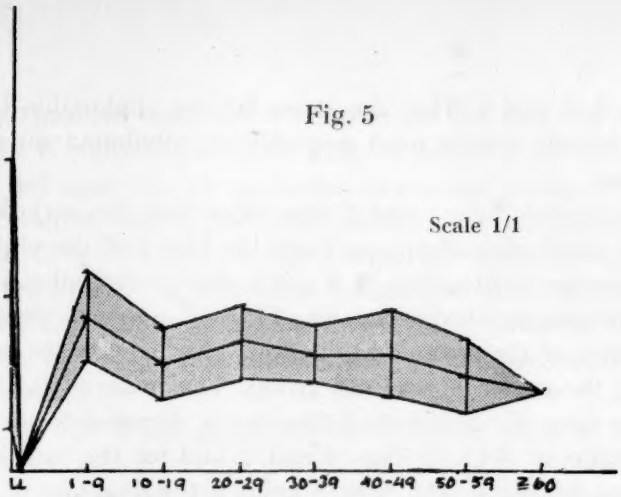
Scale 1/2



Graphic representation of the mean titre values (in the logarithmic scale) of the positive DT reactions for women.

Fig. 5

Scale 1/1

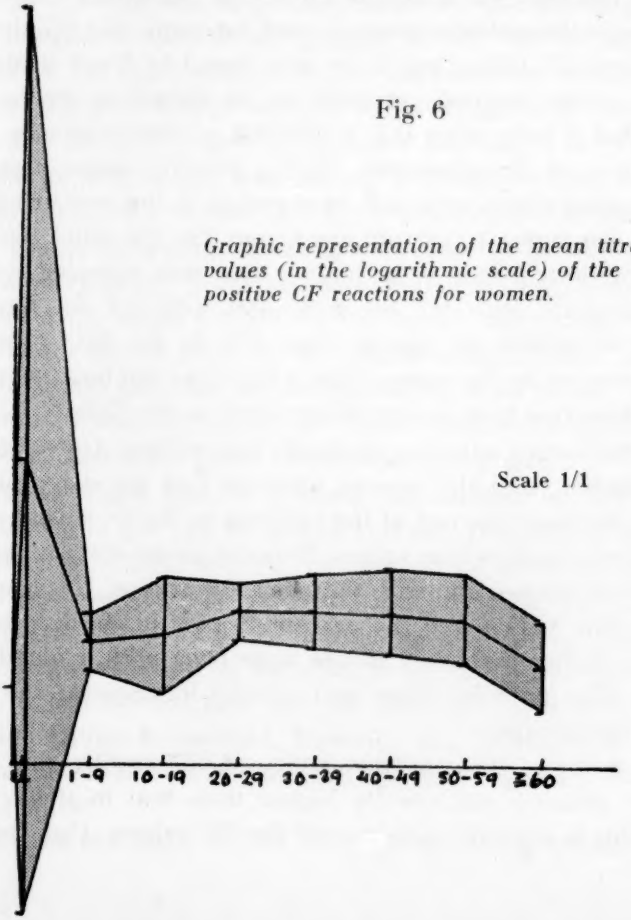


Graphic represetation of the mean titre values (in the logarithmic scale) of the positive CF reactions for men.

Fig. 6

Graphic representation of the mean titre values (in the logarithmic scale) of the positive CF reactions for women.

Scale 1/1



Figs. 3, 4, 5 and 6. That the above has no application to the CF of the female groups need probably be attributed no great importance.

Table 8 and Figs. 1 and 2 also show that the majority of DT and CF antibodies disappear from the blood of the child during the first year of life. Figs. 3, 4 and 6 show clearly that the selection principles outlined on pp. 69—71 did not give an ideal material illustrative of the normal population. This is best seen among the girls in the under 1 year old group. The material is small, and a single case of »mistaken diagnosis» is responsible for the high mean value of MTL in Figs. 4 and 6, and for the associated high standard deviation. The case in point is the daughter of a plumber's wife of 21 from the neighbourhood of Helsinki; the girl was 6 months old and was hospitalized at the Children's Clinic with the diagnosis Osteochondrosis calc. ped. lat. amb. Her DT titre was 1/16,389 and CF 1/32. Even if, as was found by Gard & Magnusson (55), a high antibody content can be shown in the newborn — they had a baby with DT = 1/2,000 — this evidently was a definite case of toxoplasmosis. In the present author's opinion, Fig. 5 illustrates best, what can be expected in the way of CF antibodies in the under 1 year old age group. On the other hand, the mean titre value seems to be higher than was expected with CF and particularly with DT for girls, and with DT for boys; the standard deviations are also great in DT. In the boys group this is partly caused by the serum from a two-year old boy from North Finland who had been under observation in the Children's Clinic. He was discharged with the diagnosis Imbecillitas. He would have been excluded from the present material had the diagnosis been available in time. The rest of the children in the 1—9 year old age group shows no high titre values. This age group was employed as the basis for comparison with the other age groups. Unfortunately, both the titre values and the percentages in this basic group are doubtless higher than they would have been with a blood donor material. This must be taken into account in assessing the results in the other groups.

Table 8 shows that the percentage of DT positives in the 20—29 age group is statistically higher than that in the 1—9 age group. This is not true, however, of the CF values. Also, the num

ber of DT-positive men and women in all the over 20 year old age groups is statistically higher than in the 1—9 age group. But this does not apply for CF antibodies to any age group. Similarly, no age group of over 1 year olds contains a statistically higher number of CF-positives if the age groups are compared mutually. This is contradictory to the results obtained by other authors with Westphal's antigen. It should be emphasized, however, that the CF-positive in the present material are over-represented in the younger age groups.

The mean age of age group 10—19 is much closer to 20 than to 10.

With age group 20—29 as the basis, significantly more DT-positive men are found in age groups 50—59 and $\geqq$ 60, but not in the 30—39 and 40—49 groups. Similarly, there are significantly more positive women in age groups 30—39, 40—49 and 50—59, but not in the age group $\geqq$ 60. By contrast, the number of DT-positive, whether men or women, is not significantly lower in age group 30—39 than in the older group s. Beverley et al. (14 a) made a similar finding in that they did not have significantly more positives in age froups of over 20 year-olds compared mutually.

The ability to develop CF antibodies seems to be the same in all age groups of over 1 year-olds. The present author cannot explain why the MTL value for DT in the female age group 40—49 is significantly higher than for the neighbouring groups, and significantly higher than for the corresponding male group. The same applies to the male age group 50—59 as compared with the adjoining groups and the women of the same age group.

Hence it seems that in Finland the number of DT-positives increases up to the age of 30 and remains practically unchanged after that age. However, the number of CF-positives reaches a certain level earlier (10—19 years) and then keeps at that level. Provided that the DT and CF antibodies are specific, and accepting the argumentation in the chapter on the persistence of antibodies, it can be assumed that the incidence of infection in the older age groups is constant. However, the incidence of infection is no higher than would balance the loss of persons who »lose» their CF titre.

This does not apply to the DT antibodies which, even if their

level is lower, persist for a longer period and thus help to increase the number of DT-positives with advancing age.

The percentage of sera with a high titre in the age groups can be seen from Figs. 1 and 2. These figures give some idea of the infection incidence per group. It seems to be of the same order in most of them, however generally so that there are more infected women per age group. Sera with a high titre for CF antibodies are only found in the age group 20—49. It is possible that this is an expression of maximally effective antibody production; it is poorer in the younger and older age groups. The persons with really high antibody formation seem always to be women, an observation also made by Harboe (66). The present author found 5 cases with a DT titre $\geq$ 1/16.389 and a CF $\geq$ 1/128, all of them women.

That the age group 20—39 contains quite a number of sera with an anticomplementary or a non-specific reaction is doubtless due to the regrettable fact that some of the sera of these age groups were stored for a lengthy period before they were CF-tested.

Age Group 20—39.

To ensure that the further investigations of the serological epidemiology of toxoplasmosis were based on a material serologically as homogeneous as possible only the sera of the 20—39 age group were employed. The material treated previously included a considerable number of mothers of children with malformations, mothers who had had abortions etc. These women, and also the women who had experienced normal childbirth, were excluded from this age group in order to make the material more homogeneous. The exclusion was made after it had been found (see p. 96) that the toxoplasmic titre and possibly also the incidence of serologically toxoplasma-positive may be higher among pregnant and puerperal women.

Table 9

Number of DT- and CF-positives in the 20—39 age group.

		No. of tested	No. of positive	Per cent positive	
Men	DT	168	34	20.2	± 3.1
	CF	251	30	12.0	± 2.0
Women	DT	264	64	24.2	± 2.6
	CF	315	45	14.3	± 2.0
Men and women	DT	432	98	22.7	± 2.0
	CF	566	75	13.3	± 1.4

INCIDENCE OF TOXOPLASMOSIS IN RURAL AND URBAN DWELLERS

There are extremely few published reports in which the urban and rural population of a single district are compared by the same authors.

Piekarski (127) found that there were fewer DT-positives (7 per cent) in the city of Bonn than among the rural population around the town (20 per cent). Roth & Fritz (136) reported that there was a higher number of positives in the university towns of Switzerland than in the Swiss Alps. This report was based on 207 sera examined, the material was small, and the university towns were over-represented. Krieg (98) found, in a material of patients with eye diseases, that the incidence of DT-positive among the inhabitants of big towns was the same as among the rural population. But there were fewer positives among patients from small towns; however, the difference was statistically significant only for the titre $\geqq 1/25$.

Westphal (172) reported for Hamburg an infection index of 2—3 per cent (DT). He later gave for a mixed urban and rural population in North Germany an infection index of 18 per cent (CF). This may suggest that the rural population has a higher number of serologically toxoplasma-positive.

Harboe (66) found 17 per cent DT-positives in a material of healthy blood donors from the city of Oslo. In a smaller material of persons from outside Oslo the percentage of positives was 10.

Kozar (97) reported that less than 20 per cent of the inhabitants of the city of Gdansk were ST-positive. However, women from a district where toxoplasma was believed to occur showed an ST percentage of 40.4.

Present Investigations

The previous chapter showed that the number of serologically toxoplasma-positive increases with age. To ensure that the further investigations into the distribution etc. of toxoplasmosis in Finland should be based on as homogeneous a material as possible, only the 20—39 age group has been employed. Table 9 gives the infection index for the whole of this group.

Table 10 shows the same material classified by urban and rural dwellers. The blood donors in the groups are treated separately.

Table 10.

Distribution of DT and CF tests of the 20—39 age groups by place of domicile in town or in country.

			Men						Women					
			No. of tested	No. of positive	% of positive	A	DT positive > 256 (%)		No. of tested	No. of positive	% of positive	A	DT positive > 256 (%)	
Town	NM	DT	82	18	22±5		1	(1)	150	39	26±4		1	(0.7)
		CF	136	16	12±3	2			178	28	16±3	9		
	BD	DT	53	8	15±5		0		70	9	13±4		0	
		CF	80	5	6±3	1			75	8	11±4	2		
Country	NM	DT	88	16	18±4		2	(2)	114	25	22±4		5	(4)
		CF	117	14	12±3	2			137	17	12±3	16		
	BD	DT	24	3	12±7		0		30	7	23±8		1	(3)
		CF	25	2	8±5	2			33	3	9±5	1		

NM = Normal material.
BD = Blood donors.
DT = Dye test.
CF = Complement fixation test.
Column A shows the number of sera that reacted anticomplementarily or against egg-protein.

Table 10 shows that, statistically, there is no difference between any of the DT groups of the table if they are compared mutually or with the entire 20—39 age group (see p. 81). The same applies to the CF reactions.

Krieg (98) found more DT positive among the inhabitants of big cities than among those of small towns. To study the state of affairs in Finland, the 20—39 age group was sub-dividad and the inhabitants of Helsinki and of the small towns with populations under 50,0000 were distinguished. For the purposes of a special

study*) sera from e.g. healthy puerperal women have been collected from Turku (pop. 106,556) and its surroundings. Although this group is not comparable with the 20—39 group (see p. 81). which contains no pregnant women or newly delivered mothers, it is included in Table 11.

The result of this investigation is shown in Table 11.

Table 11

A comparison of the DT and CF reactions of persons living in a town with 400,000 inhabitants with those of persons living in towns with less than 50,000 inhabitants; a group of women in puerperium from a town with 100,000 inhabitants (age groups 20—39) is included.

		Men			Women		
		No. of tested	No. of positive	% of positive	No. of tested	No. of positive	% of positive
Helsinki	DT	45	9	20±6	131	33	25±4
NM.	CF	86	11	13±4	131	15	11±3
Helsinki	DT	34	6	18±7	59	14	24±6
BD.	CF	60	5	8±4	59	5	8±4
All the small towns							
together	DT	29	7	24±8	31	11	35±9
NM.	CF	38	4	11±5	30	5	17±7
All the small towns							
together	DT	16	2	12±8	15	3	20±10
BD.	CF	23	0	0	15	1	7±6
Turku*)	DT				38	18	47±8
	CF				39	9	23±7

*) The sera were collected by Dr M. Takala, of Turku. They belong to a material which will be published by Takala.

NM = Normal material.
BD = Blood donors.
DT = Dye test.
CF = Complement fixation test.

Table 11 shows that, statistically, there is no difference between any of the DT groups, apart from the Turku material, if they are compared mutually or with the means of the whole age group. The same applies to the CF reactions.

The results with the material collected from Turku were surprising. It includes statistically more DT-positives than any female group whatever of Table 11, and there are statistically more positives than among the women of age group 20—29 (see p. 81). On the other hand, there is no statistical difference between the CF-positive in the Turku material and the female groups of Table 11 or those listed on p. 81. This might be interpreted to mean that pregnancy increased the number of DT-positives in the Turku material. Unfortunately no control material of healthy men or non-pregnant women is available from Turku. But, as will be seen from a later chapter, the percentage of positives among pregnant women from other parts of the country is lower than the figure for Turku.

It is a curious fact that the CF values are not as markedly high in the Turku material. This may be assumed to suggest: (1) a recently developed or already fading centre of infection in the Turku district, (2) some other infectious disease giving DT-positive DT reactions, (3) a technical error which always repeats itself in the sera from Turku. In the last-mentioned case, e.g. small quantities of detergents of invert soap type might possibly be the cause of the higher percentage of DT-positive than CF-positive. This was investigated, however, and it was found that all the tubes were washed in the same way, without invert soaps. No »non-specific» reason could be found for the high percentage, in the Turku material.

It would not be surprising, however, if one or several localities, perhaps very limited, happened to be centres of endemic or epidemic toxoplasmosis. The investigations referred to previously (p. 52) by Feldman (42) and Keller & Vivell (93) into the environment of toxoplasmic cases suggest that this may be the case. Hence geographically small areas might well be centres of toxoplasmosis (see also Kozar, Table 7). The appearance of such a centre depends on the animal population of the area and on the degree of its toxoplasmic infection. The present investigation ma-

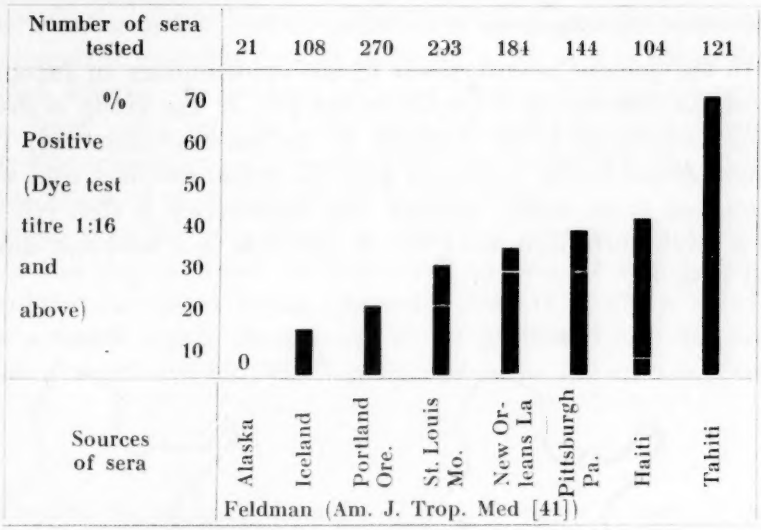
kes no great contribution towards the solution of this problem. The factor responsible for the high percentage of positives in Turku is not clear. It is hardly likely that a »toxoplasmic centre» can be detected before the infection and contagion mechanism of toxoplasmosis has been established; at present, at least, serological methods cannot perform the task.

GEOGRAPHICAL DISTRIBUTION OF THE 20—39 AGE GROUP

As was pointed out in the chapter on DT, the serologic investigations of DT by different researchers are not comparable. The same applies, to a certain extent, to CF also. The CF antigens are very different, and the borderline between a positive and a negative test is differently indicated by different authors. ST might be assumed to give more homogeneous results as the test technique itself cannot be varied very much. The fact that different toxoplasmin preparations are employed and that the concentrations of the preparations used by the different authors vary a great deal (see also p. 60) adds to the complications of comparisons.

For natural reasons, it has as a rule been impossible for any one author to investigate sera from different parts of the world. But the results available to date do permit certain conclusions. It is assumed that toxoplasma is more common in the south. Kostman (cit. by Gard, 52) reports a 10 per cent incidence of ST-positives in Boden, North Sweden, while Gard (52) found over 50 per cent positive in Stockholm (in this case the toxoplasmin employed was evidently the same). To what extent these two investigations are comparable cannot be stated.

Feldman (42) is probably the only one to have studied sera submitted from various parts of the world. This material has not yet been published in toto (42). A preliminary report appeared in Am.J.Trop.Med. (41). It is included in the table below.



Comparative incidence of antibodies for toxoplasma among 8 »normal» population groups.

The table shows that no correlation exists between the serologic incidence of infection and the points of the compass. Feldman (40) points out in another content, by way of casual note on his report, that there are very few dogs in Iceland, and that over 90 per cent of the children of Tahiti have toxoplasma antibodies at the age of 4 even. Only 2 out of 115 2—40 year old Navajo indians examined — they live in very primitive conditions — had antibodies.

Krieg (98), who examined patients in West Germany and Berlin with inflammatory eye diseases, classified them according to their domicile. It turned out that Lower Saxony and Schleswig-Holstein had the highest incidence of positives (64.1 per cent) and the North Rhine district and Westphalia the lowest (40.8 per cent). Statistically, too, there is a difference between the highest and lowest incidences. Krieg does not attempt to speculate on the cause of the differences in incidence.

The investigations by Feldman and Krieg, and that by Roth & Fritz (136) are, as far as is known, the only ones dealing with the epidemiology of toxoplasmosis on a broader geographical basis.

Present Investigations

In the present investigations of the epidemiology of toxoplasmosis in Finland the material on the 20—39 age group is distributed according to the domicile of the persons examined. This investigation is not concerned with the urban dwellers who were discussed in an earlier chapter. The distribution is first effected in a south-north direction (Fig. 7) and then in a west-east direction (Fig. 8).

Fig. 7

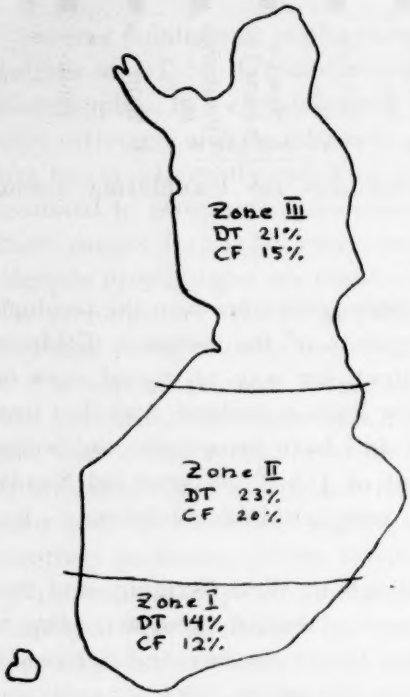
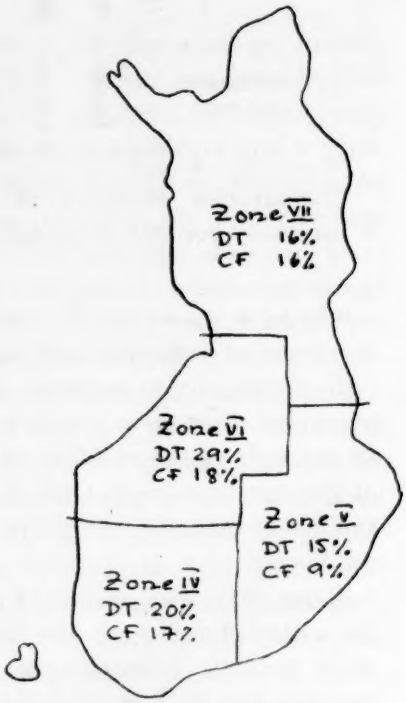


Fig. 8



Latitudes were used as the horizontal and the estern longitudes 25° and 26° as the vertical borderlines. The material of age group 20—39 was too small to allow division of the country into smaller districts. Extremely few sera were available from the northernmost parts of zones III and VII.

The result of this investigation is shown in Tables 12 and 13. Tables 12 and 13 reveal that, statistically, there is no difference

in the number of DT- and CF-positives in the groups if the groups are compared mutually or with those on p. 81. It was impossible to check whether the positives included persons who had migrated from other zones into the zone involved. However, it is hardly likely that toxoplasmosis is serologically less frequent in the north, as was assumed to be the case in Sweden (52).

Neither does this geographic investigation provide any indication of the way in which toxoplasmosis spreads. It is surprising to find that the city of Turku, situated in Zone IV of Fig. 8, should show a much higher percentages of DT-positives than the surrounding rural district. The conclusions to be drawn are given on p. 85.

Distribution by place of domicile of the DT and CF tests of the rural population in the age group 20—39

Table 12

Place of domicile situated in the following sout-north zones of Finland.

Part of Finland		No. of tested	No. of positive	% of positive
South zone.	DT	44	6	14±5
I	CF	60	7	12±4
Middle zone.	DT	52	12	23±6
II	CF	55	11	20±5
North zone.	DT	48	10	21±6
III	CF	47	7	15±5

The zones are shown in Fig. 8.

Table 13

Place of domicile situated in the following east-west zones of Finland.

Part of Finland		No. of tested	No. of positive	% of positive
Southwest zone	DT	46	9	20±6
IV	CF	59	10	17±5
Southeast zone	DT	20	3	15±8
V	CF	33	3	9±5
Middle-west zone	DT	41	12	29±7
VI	CF	44	8	18±6
Northeast zone	DT	19	3	16±8
VII	CF	19	3	16±8

The zones are shown in Fig. 8.

INCIDENCE OF TOXOPLASMOSIS IN DIFFERENT OCCUPATIONAL GROUPS

Krieg (98) has classified the patients with inflammatory eye diseases examined by him into occupational groups. He found more DT-positives among the rural population than among the others. The material was small and selected, and so perhaps cannot be considered to provide an idea of the »social epidemiology» of toxoplasmosis. Other authors have studied very special occupational groups, with no regard to social status, e.g. veterinary surgeons (127) or groups limited in some other way, as e.g. dog owners (28, 120). Usually the groups selected have been in contact with animals. This of course is natural as toxoplasmosis is considered a zoonosis (86).

How wide-spread toxoplasmosis is in the animal kingdom is uncertain. A number of the investigations was made with DT and hence suffers from the errors pointed out before.

Siim (151) showed that 10 sera from 54 dogs living in the city of Copenhagen were DT-positive $\geqq 1/256$. Otten et al. (120) found that 49 out of 122 selected dogs had positive reactions. On the whole, they considered that 2—5 per cent of non-selected dogs have a positive reaction to DT with titres $\geqq 1/25$. In addition, they found that 23 out of 38 dog owners were serologically toxoplasma-positive. Cole et al. (28) showed also that owners of toxoplasma-infected dogs were positive more often than the corresponding controls. Piekarski (127) found 32 per cent of the calves examined by him were DT-positive, and that 43 per cent of cattle and 50 per cent of sheep showed positive reactions. Thalhammer (159) found that 5 out of 34 cows were positive. He assumed that the toxoplasma could spread through cow's milk. Miller & Feldman (112) quoted a high percentage of positive dogs and goats, a somewhat lower infection index for guinea-pigs, pigs and cats, and the lowest for cattle and rabbits. Cole et al. (28), however, found over 70 per cent of cattle in two different districts to be DT positive ($DT^+ \geqq 1/2$). In certain cases they were able to show toxoplasmata in their animals. They stated that both the incidence and the titre level were lower in 67 »random samples» from a total of 400 cows. They showed experimentally that the titre of

cattle was low and assumed that their reaction is poorer than that of man and dog. They also showed parasitologic toxoplasma in epizootics of pigs, sheep and dogs.

Hülphers et al. (82) showed toxoplasma in 27 out of 840 hares that had died of natural causes. Wiktor (181) reported a toxoplasmic epizootic with high death rate in rabbits. Epizootics have also been reported in pigeons by Wiktor & Winsser (181). Marotel & Pierron (109) showed that, at a local breeding station, toxoplasma was responsible for 30 per cent of the deaths of the rabbits kept. Christensen & Siim (27) showed that toxoplasma in hares has an acute and fatal course. 9.4 per cent of the 2,812 hares they examined showed signs of toxoplasmosis. They observed that the incidence of infection was highest in the winter months. Erichsen & Harboe (38) described a toxoplasmic epidemic in White Leghorns.

The above investigations suggest that persons who come into contact with meat from animals with latent or acute infection can be expected to have toxoplasmosis more often. Kudicke & Pöhlig (99) made a DT investigation of persons handling meat products and found the group to contain 83.7 per cent DT-positive; a control group had 75.7 per cent positive. Beverley et al. (14a) examined the following occupational groups: (1) rabbit trappers, (2) rabbit handlers, (3) veterinary surgeons, (4) abattoir workers, (5) a control group. They found that the serologic incidence of the infection decreased in the order quoted. They concluded: »There is a strong correlation between contact with wild rabbits and the presence of antibodies to toxoplasma. This correlation is probably true, because it is difficult to think of any causative factor common to workers in markets and to trappers in the open.» And »Toxoplasma is possibly transmitted from rabbit to rabbit by an ecto-parasite. This ecto-parasite may survive in the fur of the animal after it has been killed.» In this connection it must be pointed out that numerous experiments have failed to show conclusively that toxoplasma is transmitted via arthropods (see e.g. Jakobs, 84). French authors have shown that the toxoplasma can occur as a natural »infection» in ticks (58) and in mites (59).

Present Investigations

In the following investigation, efforts have been made to classify the material from the 20—39 age group according to the »social status» of the persons concerned. This was done as follows: (1) persons with an academic degree, (2) those engaged in lighter occupations, (3) those employed in trade and commerce, (4) those engaged in agriculture, (5) those serving communications, (6) those engaged in industry, (7) manual labourers, (8) unspecified. The author is fully aware that this classification may not be the best from the epidemiologic point of view. But any other classification would also be incomplete. A basis of classification in corresponding investigations into tuberculosis was, for example the consumption of meat. The above classification was not always strictly observed to reflect a person's social position best, e.g. executives from groups 3, 5 and 6 have been transferred to group 2 or 1, etc. The results of this study are given in Table 14.

Table 14.

Distribution by occupation of DT and CF tests of the 20—39 age group.

	Occupation groups		Men				Women			
			No. of tested	No. positive	% of positive	DT positive >256 (%)	No. of tested	No. positive	% of positive	DT positive >256 (%)
I	NM	DT	75	14	19±5	0	72	10	14±4	2 (3)
		CF	80	7	9±3		73	7	10±3	
	BD	DT	69	12	17±5	0	59	7	12±4	0
		CF	73	5	7±3		61	6	10±4	
II	NM	DT	15	3	20	1 (7)	80	26	33	1 (1)
		CF	45	7	16		93	15	16	
	BD	DT	3	0	0	0	39	11	28	0
		CF	4	0	0		43	8	19	

(cont.)

Table 14 cont.

Occupation groups			Men				Women			
			No. of tested	No. positive	% of positive	DT positive >256 (%)	No. of tested	No. positive	% of positive	DT positive >256 (%)
III	NM	DT	4	1	25	0	42	13	31	1 (2)
		CF	7	1	14		43	8	19	
	BD	DT	1	0	0	0	21	5	24	0
		CF	1	0	0		16	6	38	
IV	NM	DT	21	5	24±9	2 (10)	67	20	30±6	2 (3)
		CF	28	5	18±7		69	11	16±4	
	BD	DT	0	0	0		0	0	0	0
		CF	0	0	0		10	0	0	
V	NM	DT	4	1	25	0	41	14	34	1 (2)
		CF	8	1	12,5		36	8	22	
	BD	DT	0	0	0	0	14	3	21	0
		CF	0	0	0		12	2	17	
VI	NM	DT	14	3	21	0	58	18	31	2 (3)
		CF	26	2	8		55	6	11	
	BD	DT	0	0	0	0	22	5	23	1 (5)
		CF	1	0	0		20	1	5	
VII	NM	DT	34	9	26±8	0	222	37	17±2	3 (1.3)
		CF	38	5	13±5		134	24	18±3	
	BD	DT	2	0	0	0	37	11	30±8	1 (3)
		CF	3	0	0		35	6	17±6	
VIII	NM	DT	13	1	8	0	36	12	33	2 (6)
		CF	6	0	0		47	11	23	
	BD	DT	11	0	0	0	5	0	0	0
		CF	0	0	0		8	2	25	

Referred to groups I, II, III . . . see text above.

NM = Normal material.

BD = Blood donors.

DT = Dye test.

CF = Complement fixation test.

A classification of age group 20—39 into as many »social» groups as were employed resulted in a small number of sera per group. It would have been tempting to combine some of the groups into a larger group, but the principle of combination is not easy to find. Groups 1, 4 and 7 are of particular interest as the standard deviation of the percentage is given for them. A marked difference in the way of life and social standing really seems to obtain between Groups 1 and 7, and if such factors affect the incidence of toxoplasmosis this difference should be visible in the infection index for these groups. In spite of the tendency towards a higher number of positive reactions in Group 7, however no statistical difference obtains between these two groups. Hence the present investigation provides no evidence of increased risk of infection in certain social groups.

In the introduction to this chapter it was shown that occupational groups in close contact with animals or meat products can be expected to show a higher incidence of toxoplasmosis. From this point of view, Group 4 is of special interest. Those engaged in agriculture in Finland are in close contact with cows, horses, sheep, poultry and often, in addition, with dogs and cats. As farmers are frequently landowners and hunt on their lands they come into contact with game more than the average population in Finland.

I is found that the number of positive serologic reactions for men in Group 4 is statistically no higher than in e.g. Group 1 or the groups on 81. By contrast, there are statistically more DT-positive women in Group 4 than in Group 1, but this does not apply to Group 4 relative to the corresponding groups on p. 81.

Even if the material seems to suggest a greater risk of infection for the agricultural population this risk is not much higher than that for any other group included in Table 14.

INCIDENCE OF TOXOPLASMOSIS AMONG WOMEN

As pointed out in an earlier chapter, the sera from pregnant women and women in puerperium were excluded from the to-

tal material order that the comparison group of 20—39 year-olds might best correspond to the normal population.

The reason for this was that Aresin (6), to mention one author, says that the incidence of positive reactions among women who have had a normal delivery is unusually high. Vivell et al. (191) and Buhn (20) claim, however, that pregnancy, in 10—15 per cent of pregnancies, raises an already extent titre. They arrived at this conclusion because a group of women who had been normally delivered revaled an incidence of DT-positives no higher statistically than that of non-pregnant women if the titre value 1/2 was taken as the limit; if the limit was placed at 1/25, however, the difference was statistically significant. The same relationship is also observed from two tables in Aresin's work (6): if 1/5 is taken as the limit, the ratio normal pregnant women: normal adults equals 1.7, but if the limit is 1/25 the ratio is 4.8.

The possible causes of the higher percentage of positives among women who have experienced a pregnancy will not be discussed here; the existence of this rise is enough to make the determination of DT and CF reactions in pregnancy more difficult still. The same applies to the examination with these tests of various groups of delivery with complications, etc. One of the focal points of toxoplasmosis research consists of investigations into the significance of toxoplasmosis as the factor responsible for abortions, stillbirths and malformations. This problem has not been solved, but the optimistic attitude sometimes adopted, for example that a latent toxoplasmosis of the mother is the factor responsible for a large number of stillbirths, is certainly incorrect (179). Similarly, it has not been ascertained whether it is only an acute infection in the mother that results in toxoplasmosis of the child. The problem in general is difficult to solve. On the one hand, there is the clear and typical picture of congenital toxoplasmosis. This is doubtless rare. Bamatter (12) found no single instance of toxoplasma among 6,000 newborn infants. According to Vivell & Buhn (191) 1 pro mille of all newborn are toxoplasmic children. Strobel (156) claims that manifest toxoplasmosis in children is a rarity.

There is no doubt that toxoplasmata can live in excellent equilibrium with the host organism. According to the principle that nature never advances in leaps there should exist a number of

infection conditions, more or less serious from the human point of view, between congenital toxoplasmosis and a toxoplasmic pseudocyst in a reaction-free environment. Whether and to what extent these conditions are responsible for abortions, stillbirths and malformations continues to be a taxing problem.

The most natural course has been approach the problem by the serologic route.

Zilliacus & Wist (195) examined 163 women who had abortions, stillbirths and different complications during puerperium. 45 of them were serologically positive. 6 out of 19 women (32 per cent) who had had abortions and macerated infants were serologically positive, 11 out of 37 women (30 per cent) with prematurely-born infants and 3 out of 7 women (43 per cent) who had had recurrent abortions were positive. On the other hand, among 26 cases of habitual abortions there were only 5 (20 per cent) with positive reactions.

Holmdahl's investigations, reported in Table 7 on p. 62, are of interest in this connection. The same table also contains references to investigations by Zeipel & Lindner, Buhn, and Garin. In addition, Kirchoff & Kräubig (cit. by Aresin [6]) examined 23 miscarriages of which 19 were DT-positive, and 18 stillbirths of which 12 were DT-positive. They were unable to show toxoplasma parasitologically. Aresin's (6) work revealed that 276 DT-examined children of a material selected from a total of 10,000 births included only 25 per cent positive $\geqq 1/5$. This is surprising in that the number of positives is smaller than in the control material of cases of normal delivery.

Present Investigations

The present material also includes various groups of women who had had abortions etc. The classification of this material had to be based on the diagnoses recorded on the labels accompanying the sera submitted. Hence the classification is very incomplete. Classification within a hospital, with the »standard diagnoses» of the hospital, is difficult enough; the present material comprises sera from various hospitals and general practitioners in Finland and is therefore very heterogeneous. For comparison's sake, only

sera from the 20—39 age group have been dealt with. The results are shown in Table 15.

Table 15.

Classification of women in the age group 20—39.

	Groups		No. of tested	No. of positive	% of positive	DT positive > 256
I	Women in puerperium with normal children	DT	122	27	22±4	0
		CF	127	23	18±3	
II	Healthy non-pregnant women	DT	44	10	23±6	
		CF	46	6	13±5	
III	Women who have had abortions	DT	24	2	8±6	0
		CF	25	0	0	0
IV	Women with abortus habitualis	DT	22	5	23±9	2
		CF	25	3	12±7	2
V	Women who have had stillbirths	CF	60	6	10±4	1
		DT	54	15	28±6	0
VI	Women who have had a monster	DT	19	5	26±10	1
		CF	20	2	10±7	0
VII	Mothers of children with developmental defects.*) (From the town of Turku)	DT	108	48	44±5	
		CF	110	25	24±4	
In group VII are included mothers of children with:						
	Meningo-myelocele	DT	9	4	44	
		CF	10	1	10	
	Hydrocephalus	DT	19	6	32±11	1
		CF	19	3	16±8	
	Anencephalus	DT	8	3	38	0
		CF	8	2	25	
	Idiotia mongoloida	DT	20	10	50±11	0
		CF	20	5	25±10	

*) These sera are also included in a work by M. Takala, Turku.

The table shows that, statistically seen, no difference exists between Groups II, III and IV if the groups are compared mutually or with the female group on p. 81. Group II comprises women

who have never been pregnant. It is surprising that Group I (abortions) reveals statistically fewer DT-positives than the other groups. A similar finding was made by Zilliacus & Wist (195). How this is to be interpreted is unclear. Even serologic insufficiency of the mother may be involved. Group II (habitual abortion) is particularly heterogeneous in that it includes also recurrent abortions of a nature other than »habitual».

A comparison of Group VII with the other groups shows that it contains a higher number of serologically positive than Groups I—V and the groups on p. 81. Groups VI and VII are comparable; all the sera of Group VII, however, derive from one district. In spite of the tendency towards higher positive percentages in Group VII, there is no statistical difference between it and Group VI.

However, on comparing Group VII with cases of normal delivery from Turku (see p. 84) no difference is found. This emphasizes the fact that only sera from one and the same district can be employed as control material in different special investigations. The present author, therefore, prefers not to compare his material with previous investigations.

The author does not believe that the problems under review can be explained solely by serologic methods as long as our knowledge of the serologic tests is incomplete.

SUMMARY

Section I shows that, with our present knowledge of the toxoplasma, it is impossible to classify the organism.

Before the distribution and significance for man of toxoplasmosis can be made clear the infection mechanism apparently must be verified; the classification connected with this will then inevitably be solved.

Section II reveals that the serologic methods of diagnosing toxoplasma, especially the dye test, are non-specific. They are therefore less suitable for studies of the serologic epidemiology of toxoplasmosis until we know the degree of specificity that can be ascribed to these serologic methods. Hence it is necessary

a) to study the serologic mechanism in the dye test and the bio-

logical significance of the cytoplasm-modifying antibodies,

b) to carry out long-term investigations into the antibody variations in parasitologically verified cases of toxoplasmosis.

Section III shows that the index of toxoplasma infection in Finland, for age group 20—39 measured with the non-specific tests. dye test and complement fixation reaction, is 22.7 ± 2.0 and 13.3 ± 1.4, respectively. The present investigation seems to indicate a local centre of toxoplasmosis in South-West Finland. The risk of infection in the different age groups is approximately equal; the same applies to the different social groups and to urban and rural dwellers. Certain occupational groups may be more exposed to infection; however, this was not revealed by the present study. It is also evident from the present investigation that the control material in serologic investigations of certain disease groups must be collected from the districts in which the diseased are domiciled.

The fact that the serologic tests proved non-specific makes it impossible at the moment to supply definite information on the distribution of toxoplasmic infection. It is possible that the incidence of infection has been considerably overestimated. However, it must be borne in mind that toxoplasma seems to possess every possibility of being a micro-organism extremely commensal with man. It is possible that the strains isolated to date and employed in the laboratories are unusually virulent. The ability of toxoplasma to live extremely commensally makes it an exceedingly interesting microorganism even though its distribution may not be very extensive.

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